Hospital Library

JUL 21 1930

PHYSIOLOGICAL REVIEWS

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

	PAGE
THE INTERCONVERSION OF THE MAJOR FOODSTUFFS. *David Rapport*	349
THE RÔLE OF THE SMALL INTESTINE IN NUTRITION. *H. E. Magee*	473
HEMOGLOBIN, THE HEME PIGMENTS, AND CELLULAR RESPIRATION. *M. L. Anson and A. E. Mirsky*	506

VOL. X, No. 3

Issued July, 1930

BALTIMORE, U. S. A.

Entered as second-class matter, January 15, 1921, at the Post Office at Baltimore, Md., under the act of March 8, 1879

Made in United States of America

PHYSIOLOGICAL REVIEWS

PHYSIOLOGICAL REVIEWS is owned and controlled by the American Physiological Society. It is edited by a board appointed by the society consisting of:

W. H. HOWELL, Baltimore
LEO LOEB, St. Louis
J. J. R. MACLEOD, Aberdeen, Scotland
A. J. CARLSON, Chicago
P. A. SHAFFER, St. Louis
TORALD SOLLMANN, Cleveland
D. R. HOOKER, Managing Editor, Baltimore

The Editorial Board selects the subjects and authors of all articles which are published, the aim being to provide concise but comprehensive reviews of the recent literature and present status of various subjects in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology. Each volume will consist of from sixteen to twenty articles, making a total of about five hundred pages per volume. The numbers will be issued quarterly in January, April, July and October of each year.

Subscriptions will be received in advance only. The subscription price is:

$6.00 per volume in the United States, net postpaid
$6.25 per volume in Canada, net postpaid
$6.50 per volume elsewhere, net postpaid
$2.50 per single number, net postpaid

Subscriptions should be sent to

D. R. HOOKER, *Managing Editor*
19 W. CHASE STREET, BALTIMORE, MD.

Tentative Contents of Volume X, 1930

A. E. MIRSKY: The Hemoglobin Compounds, with Special Reference to Protein Chemistry and the Significance of the Heme Pigments in Tissue Oxidations

H. M. EVANS: The Anterior Hypophysis

J. H. BURN: The Errors of Biological Assay

T. GRAHAM BROWN: The Technique for Recording Reflexes from the Decerebrate Preparation

H. E. MAGEE: The Physiology of the Small Intestines

E. D. ADRIAN: The Action of the Sense Organs

LEO LOEB: Individuality and Transplantation

V. E. HENDERSON: The Present Status of the Narcosis Theories

W. F. VON OETTINGEN: Excretion and Distribution of Bismuth Compounds

S. W. BRITTON: Adrenal Insufficiency

BALDUIN LUCKÉ: Cellular Osmosis

DAVID RAPPORT: The Interconversion of Foodstuffs

A. B. HASTINGS: Comparative Effects of Chlorides, Bromides and Iodides on Animals

CARL F. CORI: Carbohydrate Metabolism

PHYSIOLOGICAL REVIEWS

VOL. X JULY, 1930 No. 3

THE INTERCONVERSION OF THE MAJOR FOODSTUFFS

DAVID RAPPORT

The Department of Physiology, Tufts College Medical School, Boston

Certain considerations encourage the idea of a general interconvertibility of the major foodstuffs in the mammalian organism, for example: 1. In certain instances the known ability of plants and bacteria to achieve a particular conversion induces a presumption in favor of a similar ability on the part of mammals. It becomes a pressing temptation to lean heavily upon the argument by analogy, which is dangerous here as elsewhere. 2. The tendency toward reversibility observed in bodily reactions inspires the postulate, when a certain conversion is proved, that the reaction must be able to go in the reverse direction as well. 3. Differences in the efficiency with which the body does work at the expense of one foodstuff, as compared with another, invoke assumptions on the basis of thermodynamic data that conversion from one to the other is taking place, even though the specific chemical reactions are unknown, rendering the assumptions unsafe. 4. When it is assumed that a certain foodstuff is needed for certain functions, and these functions continue to be performed in its apparent absence, it becomes inevitable to suppose that the foodstuff is synthesized, and that it may be formed from one of the other major foodstuffs.

The above considerations do not unavoidably force the conclusion that all of the foods are interconvertible. Each case has to be examined on its own merits. Even when the evidence seems to argue against any individual conversion, however, it should be emphasized that none has been finally ruled out. One cannot be unmindful of the inviting conception of Knoop: "Mit diesen chemischen Fähigkeiten (referring to such conversions) wird der Tierkörper bei einseitiger Nährstoffen vor oder vorübergehenden Mangel an einzelnen Nährstoffen vor Schaden geschützt. Er braucht Eiweisz, Kohlehydrate und Fett zu den lebenswichtigsten Funktionen. Fehlt ihm einmal das eine oder das andere, so kann er sie sich bis zu einem gewissen grade selbst aufbauen."

THE SYNTHESIS OF PROTEIN.[1] It can be regarded as well established that plants can synthesize their amino acids, and thence their protein, from simpler nitrogenous compounds. It is equally beyond question that bacteria, yeasts, and some of the more primitive forms of animal life can also do so. Soil bacteria, in fact, play an undoubted and important rôle in the preparation of the simplest nitrogenous radicals—even nitrogen itself—for plant utilization; and, as we shall see, the presence of microörganisms complicates the problem of protein synthesis in mammals.

The direct evidence in regard to the mammalian organism has been based largely on experiments with herbivora on the one hand, and omnivora and carnivora on the other. Certainly, positive evidence of protein synthesis in herbivora might reasonably be expected to presuppose a similar phenomenon in all mammals. Yet here also the value of the argument, in so far as carnivora are concerned, is in question. For it is clear that a possible distinction exists between the plant eaters and the meat eaters. Utilization of the simpler nitrogen compounds by herbivora, if it can be shown to exist, might be accounted for by bacterial intervention in the gastro-intestinal tract. The problem in this case, therefore, might seem to be simply a reversion to the question of whether bacteria can synthesize protein, and we know that they can. Yet this postulated distinction is not certain, and it is well to examine the evidence for the synthesis of protein by herbivora.

I. Experiments on Nitrogen Retention in Herbivora. To a large extent these experiments have been concerned with the question of the retention of nitrogen following the ingestion of asparagin, the latter being used because of its natural occurrence in large quantity in the vegetable foods. In 1879, and again in 1881, Weiske added asparagin to the protein-poor diet of sheep, and observed a retention of nitrogen. Thus, in one case, on the basal ration, there was a retention of 0.279 gram N; on adding asparagin to the diet, there was a retention of 1.38 grams N, a result similar to that which he obtained with gelatin. He concluded that "asparagin has a definite value in animal nutrition, and is a foodstuff in the same sense that gelatin is, in sparing protein." In revising his work in 1894, he pointed out that the protein sparing action of asparagin described in his earlier papers occurred in a protein-poor, but carbohydrate-rich diet, and emphasized the difference between the

[1] In the ensuing section, the synthesis of protein from amino acids will not be considered.

action of asparagin and that of carbohydrate, which, according to him, spares protein best when the diet contains much protein and little caloric content.

Kellner (1900) administered asparagin to lambs subsisting on a protein-poor diet and also observed nitrogen retention, but the asparagin had no effect on the already existing nitrogen retention when the animals were on a protein-rich diet.

These experiments served to indicate a utilization of asparagin by the animal, but in a series of experiments carried out independently by Völtz (1905, 1906), Friedländer (1907), and Just (1908), it was noted that on feeding plant extracts to sheep there was an increase in the protein nitrogen of the feces. This was interpreted to mean that the non-protein nitrogen of the food was being converted to indigestible protein by the bacteria of the intestine. However, on further investigation of the phenomenon it was found by Kellner and his co-workers (1910), as well as by Morgen and his collaborators (1910) that while there was an increase in the fecal protein nitrogen on feeding plant extracts to herbivora, this was not true in the case of asparagin and ammonium salts, and that even in the former case, the increased fecal nitrogen was pepsin-soluble, and was to be attributed to an increased secretion by the cells of the intestinal mucosa.

In addition to these experiments with asparagin, there were some in which other non-protein substances were employed. Kellner, Eisenkobbe, Flebbe, and Neumann (1910) observed a retention of nitrogen in lambs on feeding ammonium salts; Völtz (1905) had noted a gain of 15 grams of protein daily on administering urea. (This observation by Völtz was later challenged by Salkowski (1920) who stated that the sulphur of the retained protein could not have been supplied by the diet.)

On the face of it, these experiments appear to make it clear that in herbivorous animals, at least, there is a retention of nitrogen under the conditions mentioned. But before this can be accepted as implying an ability on the part of the animal to synthesize protein, two questions must be answered: 1. Does nitrogen retention, *per se*, imply protein synthesis? 2. Is the utilization of the ingested nitrogen not due entirely to bacterial action? As to the first of these questions, I shall postpone its discussion until later. As to the second, which has already been touched upon, it was first clearly pointed out by Zuntz (1891) that if differences existed between utilization of non-protein nitrogenous substances by carnivora and herbivora, it could probably be ascribed to the fact that bacterial action was much more likely to occur in the intestines

of the latter. He advanced the hypothesis that the non-protein substances were synthesized to bacterial protein in the gut of a herbivorous animal, thus "protecting" the protein of the food, and being itself later digested and used by the animal. This conception of bacterial action was held, in a general way, by the later workers who obtained positive instances of retention, though Armsby (1911) was led to say that after all, bacterial intervention in the case of herbivora was only a postulate, believed in simply because no adequate proof had been advanced that carnivora could change the simpler nitrogen compounds to protein. Were the carnivora shown to possess this power, the necessity for believing in bacterial intervention would disappear. Nevertheless, if nitrogen retention upon the ingestion of non-protein nitrogenous compounds by herbivora could be accepted as a fact, the experiments of Henriques and Anderson (1914) offered confirmatory evidence that it was due to the activity of bacteria in the gastro-intestinal tract. These investigators injected sugar, sodium acetate, sodium citrate, urea, and ammonium acetate intravenously into goats, and in no case found a lasting retention of nitrogen.

Whether the phenomenon of nitrogen retention in herbivora under the experimental conditions is or is not due to bacteria, the evidence, with a single exception, seems to indicate that it occurs. This exception is found in the experiments of Scheunert, Klein, and Steuber (1920), who found that if the excretion of nitrogen through the skin was taken into account, there was no nitrogen retention in lambs after ingestion of urea. These investigators offer no data in regard to other non-protein substances. Failing more evidence to the contrary, we may leave this phase of the subject with the presumption that nitrogen retention by herbivora upon administration *per os* of certain non-protein nitrogenous substances is an actual occurrence, without at present going beyond this fact.

II. Earlier Experiments upon Omnivora and Carnivora. Following Weiske's experiments on herbivora, a certain number of researches were early carried out on omnivora and carnivora, chiefly in Voit's laboratory. Munk (1883) brought dogs into nitrogen equilibrium on either an exclusively protein or a mixed meat and carbohydrate diet. On adding asparagin (as much as 1 gram per kilo per day) there was no evidence of nitrogen retention and the sulphur excretion actually indicated an increase in the protein metabolism. Politis (1891), working with rats, noted the loss of weight and the time it took for the animals to die on a nitrogen-free diet. Adding asparagin to this diet did not markedly

affect these factors. Still, there was some little evidence of a sparing action on protein and fat metabolism, as there had been in Weiske's experiments, though Politis himself laid no emphasis on this. Mauthner (1891), using dogs, also obtained a very slight sparing action with asparagin, again using the body weight as a criterion. Gabriel (1892) criticised the conclusions of Politis, and pointed out that in the latter's experiments asparagin must have had some nutritional value, for granting nearly equal loss of weight by the animals with and without asparagin, less carbohydrate and fat must have been consumed when asparagin was added. The quantities of asparagin involved, however, make the criticism of doubtful value. Gabriel himself also used rats, and obtained definite evidence of a sparing action on metabolism. It should be noted that nearly all of these experiments used body weight as an index of the nutritive value of the ingesta, and bearing in mind the uncertainty of the criteria, not too much weight can be attached to the results. Voit (1892), indeed, in a discussion of these papers, came to the conclusion that asparagin did have some value in the nutrition of carnivora, though he regarded it as of little importance. The experiments here quoted were all carried out on protein-poor and protein-free diets, and Gabriel seized upon his positive results in such diets—negative on a protein-rich diet, as in Weiske's experiments—to postulate that the action of asparagin was simply to increase the utilization of carbohydrates, and not to replace protein. The theory was vague, and Voit declined to accept it, but offered no very definite theory of his own. Much later, Henriques and Hansen (1907) also performed experiments with rats, adding asparagin and various "amide mixtures" to an otherwise nitrogen-free diet, and could obtain no evidence of protein sparing. The continuous loss of nitrogen on the nitrogen-free diet was practically unchecked when the amides were added. They concluded that omnivora behaved quite differently from herbivora, and especially from ruminants.

It may be stated parenthetically here, that with the exception of Weiske, the experimenters upon herbivora had employed a protein-rich diet. This led to a criticism by Armsby (1911), who stated: "When the animal was in nitrogen equilibrium with the basal ration, the cleavage products arising from the digestion of protein were doubtless being oxidized to a considerable extent, and their nitrogen excreted as urea. Such being the case, while additional protein might cause more or less gain of nitrogen for a time, additional non-protein might easily produce indirectly a similar effect, without implying any formation of protein

from it, simply by taking the place of some of the cleavage products whose nitrogen was previously split off." Now in the first place, if nitrogen set free by deaminization is retained in the body, no distinction can be drawn as to the form which is retained as between protein and non-protein nitrogenous compounds. In the second place, it may be assumed that lasting nitrogen retention upon the addition of protein to the diet implies retention of protein, and if a similar retention occurred with non-protein (nitrogen compounds), it might also be considered as protein retention. True, it might not, but if so, the same argument applies in the case of the protein-poor diet, where again, nitrogen retention following protein ingestion may be considered as implying protein retention, while after the ingestion of non-protein, it may not. The point is that we have no criterion for drawing the distinction, which does not seem to me, therefore, on these grounds, to be a valid one.

Rosenfeld (1900) added finely chopped cooked hay to the food of dogs, and found an even greater nitrogen retention with this asparagin containing substance than with albumen. Müller (1907) obtained nitrogen retention with asparagin, there being a considerable difference between free asparagin and that coated with celloidin, showing the influence of the rate of absorption. Lehmann (1906) had also observed this difference, but obtained no evidence of nitrogen retention. In all of these experiments there was an ample amount of protein in the basal ration.

III. The Reversal of Oxidative Deaminization. This was the somewhat unsatisfactory state of affairs when Neubauer's discovery of oxidative deaminization led Knoop (1910) to suspect that the reaction might be reversible. With this in mind he gave α-keto γ-phenylbutyric and α-hydroxy γ-phenylbutyric acid to a dog by mouth and subcutaneously, and found that d-α-acetyl-amino γ-phenylbutyric acid was excreted in the urine. In carrying out a similar experiment with Kertess (1911), he again found on giving the keto phenylbutyric acid an excretion of a small quantity of the corresponding acetylated amino acid. These experiments led Knoop to the conclusion that the organism is able to synthesize amino acids from keto-acids and ammonia already present in the body. He postulated, as an intermediary step, a hypothetical hydroxy amino acid. A similar result was obtained by Kondo (1912) in Embden's laboratory. He perfused dogs' livers with α-keto butyric acid and α-keto-n-caproic acid, recovering the corresponding amino acid in the perfusate.

While Knoop inferred that the process he described was applicable

to the naturally occurring amino acids, the phenyl amino acid that he obtained in the urine does not exist in protein. It remained for Embden and Schmitz (1910, 1912) to test Knoop's hypothesis with amino acids that are actually present normally in the body. They perfused dogs' livers with blood to which 5 grams of oxy-phenyl pyruvic acid had been added, and obtained 0.5 gram of tyrosin, the tyrosin being recovered in the dinaphthalein-sulfo-tyrosin form. Likewise, they perfused with 5.7 grams of phenyl pyruvic acid, and found 0.5 gram of phenylalanine; and on perfusing with pyruvic as well as lactic acid, alanine was recovered. All of these experiments were carried out in glycogen-poor livers. In passing, I should mention that on adding NH_4Cl to the perfusing blood, alanine was found in the perfusate, but only in glycogen-rich livers. This result was also obtained by Fellner (1912).

Both Knoop and Embden obtained optically active amino acids, and in the latter's experiments the optical activity was the same as in the naturally occurring acids. These experiments were particularly important in that they seemed to prove for the first time that many of the amino acids of protein could be synthesized in the animal body. In stating this, the mass of evidence indicating the probable synthesis of glycine is not being overlooked. I do not propose to go into the voluminous literature on the subject here. It is sufficient to state that there is overwhelming testimony that a new formation of glycine can occur in the animal body. The weight of evidence favors the view that it arises under these circumstances as a result of synthesis in some obscure manner from simple nitrogenous compounds, and not from a breakdown of more complex amino acids, a possibility which Knoop (1914) suggested. Yet glycine had always appeared to be unique in this respect, and there was, moreover, no reason to believe that the protein molecule could be in turn synthesized from glycine.

The experiments of Knoop and Embden resulted in only very small yields of amino acids, but the fact that they were obtained at all gave a fillip to research, for it seemed to put the problem of amino acid synthesis on a more firm theoretical footing. A number of investigations followed, which I shall attempt as far as possible to present according to the nature of the substances added to the diet.

IV. Later Experiments on Nitrogen Retention in Carnivora. Völtz (1906) had given ammonium acetate to dogs, but had found no increase of nitrogen retention over that occurring on the basal ration. The first fruits of the experiments of Knoop and Embden were a flood of experi-

mental work from the laboratories of Grafe and Abderhalden, reverting to the problem of nitrogen retention upon the administration of the simpler nitrogenous compounds. Grafe adopted the hypothesis that the conclusions of Knoop were sound, and that protein could be synthesized from nitrogen in the form of urea or ammonia. He also assumed that carbohydrate could be converted in such a synthesis. Accordingly Grafe and Schläpfer (1912), keeping dogs on a high carbohydrate, low-protein intake, in such a manner that there was a negative nitrogen balance, added ammonium citrate to the diet, and not only were able to reduce the negative balance, but were even able to maintain a dog in nitrogen equilibrium. Their technique was to fast the animal for several days, then to institute a pre-period on the high-calorie, protein-free diet (containing an abundance of both carbohydrate and fat), during which the protein metabolism was at a minimum. This was followed by a period in which the non-protein nitrogenous substance was added. Then another high-calorie, protein-free after-period was instituted. The last three days of the pre-period and the first three days of the after-period were used as criteria for comparison with the period of administration of the ammonium salt. Twenty-four hour intervals were not separated by catheterization; but the results were sufficiently definite not to be invalidated on this ground. The investigators found, upon the administration of ammonium acetate, not only a reduction in the loss of nitrogen occurring in the "basal" periods, but even the maintenance of nitrogen equilibrium over a period of more than two weeks. The retention of nitrogen over this considerable time was in itself significant, but they also were unable to observe any appreciable increase in the nitrogen elimination after the completion of the ammonium acetate period. Similar, though less striking results, were obtained with ammonium chloride.

A paper by Abderhalden immediately followed this one, and for some time communications emanated from the two laboratories in rapid succession. For simplicity, those from Grafe's laboratory will be outlined first. The paper of Grafe and Schläpfer was succeeded by one from the pen of Grafe (1912, 1) reporting experiments in which a dog was kept for three months on a high-carbohydrate, protein-free diet to which ammonium citrate had been added. The nitrogen balance was nearly always negative in this period, and the dog eventually died. However, Grafe attributed this death to the absence of iron, and concluded that sufficient evidence had been adduced to show that there had been a sparing of body protein. Grafe (1912, 2) reported similar results to

those he had obtained in dogs when collaborating with Schläpfer. Working with Turban, Grafe (1913) administered urea, and as in the case of ammonium citrate, found a decrease in the negative nitrogen balance, with an occasional positive balance. On administering urea to a pig, he found that the nitrogen balance was constantly negative. After this, Grafe (1913, 1, 2) changed his method. In order to obviate to some extent the great loss of nitrogen, he added small amounts of protein to the "basal" ration, but not enough entirely to cover the protein deficit. On doing this, he found that the small negative nitrogen balance of the control periods was converted to a positive balance when either ammonium citrate alone, or ammonium citrate plus urea, were added to the diet. Under the new conditions, the constant negative nitrogen balance which he previously observed to occur when urea was fed to a pig was converted to a positive balance. The same was true of ammonium citrate (1913, 3). Grafe also published a paper (1914) in which he returned to the question of the effect of administering an inorganic ammonium salt. On giving ammonium chloride in small doses, he obtained evidence of slight nitrogen retention. With Wintz, Grafe (1914) turned his attention to nitrogen retention upon the administration of sodium salts. On giving sodium nitrate, these authors observed a retention of ten to fifteen per cent of the salt. Large doses acted toxically and increased the nitrogen excretion. They assumed that $NaNO_3$ was converted to NH_3, and in this manner utilized by the body.

The numerous experiments conducted by Grafe and his collaborators led him to the conclusion that there was undoubtedly a retention of nitrogen on administering nitrogen in non-protein form, and that this retention was conditional upon the presence of an ample supply of carbohydrate in the body. As to the meaning of this retention, Grafe mentions three possibilities: 1. That ammonia may be held in the body as such; 2. That it may be combined in a non-protein form: 3. That it may be synthesized to protein. He does not at any time insist upon the certainty of the latter, but he regards the evidence of nitrogen retention coupled with the fact that in his experiments the retention occurred on a high-carbohydrate diet, and taking into account the researches of Knoop and Embden, as favoring the view that protein is actually synthesized in the body from ammonia and carbohydrate.

I have already mentioned the earliest paper by Abderhalden on the subject (1912). Abderhalden called attention to the existing evidence indicating that at least one amino acid, namely, glycine, could be synthe-

sized, and also to the work of Knoop and Embden. But though he regarded the work of the latter as suggestive, he pointed out that this presupposed the presence of the necessary keto acids in the body, and that therefore the proof of amino acid synthesis apart from glycine was not yet at hand. Following a technique that was similar to that of Grafe, he gave to dogs for a period of a week or more a high calorie diet, containing much carbohydrate and fat, but no protein. (It is to be noted that Grafe also included fat in his diets, hence the evidence for carbohydrate intervention rather than that of fat must be qualified.) During a period of three days, he added ammonium citrate or carbonate to the diet, and observed a reduction in the negative nitrogen balance (which had been as high as almost 3 grams N) to a point where the animal was almost in nitrogen equilibrium. A considerable effect was also obtained with ammonium acetate. In these experiments, there was no "washing out" of the retained nitrogen in the period following the NH_3 ingestion. Assuming that retention occurred, Abderhalden at this time admitted the possibility of protein synthesis.

These observations were continued by Abderhalden and Hirsch (1912, 1, 2, 3, 4; 1913), who carried out long continued observations on dogs fed with ammonium salts and urea, as well as with gelatin, and mixtures of amino acids. The results are somewhat confusing and in some experiments only the body weight was used as a criterion, but in general the evidence indicated that while there was a temporary retention of nitrogen when organic ammonium salts were added to a protein-free diet, this retention was followed by a "washing out," and from the data it would appear that some lasting retention occurred. Of the organic ammonium salts, Abderhalden and Hirsch tested the acetate, valerate, lactate, citrate and tartrate. When these workers (1913) administered ammonium chloride they could find no evidence of retention as opposed to the results of Grafe and Schläpfer.

In other experiments with Lampé, Abderhalden found substantially the same results. In the dog (1912), and in the pig (1913), they administered ammonium acetate, citrate, or phosphate, and on a high calorie non-protein diet they were able to find very little evidence of nitrogen retention.

On the basis of his later experiments, Abderhalden modified his earlier views. To some extent his experimental results were not greatly dissimilar to those of Grafe and his collaborators, the chief difference being in the amount and duration of the nitrogen retention. In the case of Grafe's experiments, there was always evidence of retention, even

though it was sometimes so slight that the errors of the method made this highly questionable. Abderhalden's co-workers considered that even this retention was nearly always accounted for by a later "washing out." In summarizing their experiments Abderhalden and Hirsch (1912, 3) state: "We draw from our experiments the conclusion that ammonium salts and especially ammonium acetate are sometimes able to effect the nitrogen balance in the sense of retention. There is no foundation in our results for the belief that the animal cells can synthesize protein from ammonia and nitrogen free compounds (carbohydrates, etc.)."

Again, Abderhalden and Lampé (1912), referring to Grafe's belief that ammonium salts, urea, and even sodium nitrate can serve as a source of protein, say: "We have come to other conclusions. There is no doubt that in certain cases the nitrogen excretion can be diminished by administration of ammonium salts. We have not observed a long continued positive balance. Only in a few cases was it transitorily positive. These were afterwards all the more strongly negative. We incline to the viewpoint that the well established nitrogen retentions have no relation to protein synthesis. We have no reason to believe that the animal organism builds the scaffolding for all the variously constructed amino acids from carbohydrate, and uses the supplied ammonia for aminization."

One difference between Abderhalden's technique and that of Grafe was that Grafe gave his animals larger quantities of carbohydrate and he attributed the fact that he obtained greater evidences of retention than did Abderhalden partly to this, believing, as he did, that carbohydrate was acting as a source of protein. Abderhalden, however, did not agree with this view.

The conclusions of Grafe in regard to the retention of nitrogenous sodium salts were confirmed by Peschek (1912) who noted nitrogen retention on feeding sodium acetate. But Abderhalden and Hirsch (1912, 2) found that sodium nitrate was excreted quantitatively, in spite of the fact that in two of their four dogs the nitrogen balance was otherwise favorably affected.

The findings of Grafe were further criticised by Henriques and Anderson (1914), whose experiments on goats have already been mentioned. These negative results on injecting sodium and ammonium compounds led them to infer that the nitrogen retention noted by Grafe was due either to bacterial action in the intestinal canal, or to a simple retention of the substance itself. It is not clear why they should have drawn the

second of these conclusions, but the first, though based upon experiments with herbivora, certainly has a bearing upon carnivora as well.

Another indictment of one of Grafe's conclusions appears in the work of Underhill and Goldschmidt (1913). It will be recalled that Grafe obtained positive evidences of nitrogen retention, though slight, with an inorganic ammonium salt, NH_4Cl. Underhill had called attention to a distinction in the behavior of organic and inorganic ammonium salts in that the former were entirely changed to urea, while of the latter most of the NH_3 was excreted as such. Following this, Underhill and Goldschmidt found that ammonium chloride ingested by dogs maintained on a non-nitrogenous diet was only temporarily retained, and that this was accompanied by evidence of toxicity. They concluded that NH_4Cl, far from being a source of nitrogen for the body, is actually "a detriment to nutritional rhythm." In the case of organic ammonium salts, such as the acetate and citrate, they confirmed Grafe's results, finding a marked reduction of the nitrogen loss (on a high calorie, protein-free diet) with no evidence of a later excretion of the retained nitrogen.

G. D. Cathcart (1916) injected NH_3, urea and glycine intravenously into dogs and found an accumulation of these bodies as such in the liver and to a less extent in muscle. She concluded that there was "no evidence of the synthesis of any of these substances into protein." However, there is ample evidence that a heaping up of amino acids and urea can occur in the body during the course of protein metabolism, yet it is possible for protein to be synthesized under these conditions. So the argument of Cathcart cannot be regarded as conclusive, though it may have some quantitative bearing.

The experiments of Gessler (1920) are of importance in this connection. This investigator obtained nitrogen retention, though not a positive nitrogen balance, in starving dogs upon administering ammonium acetate and citrate. Yet the sulphur excretion was not reduced. He therefore concluded that the nitrogen retention signified that NH_3 was not protecting the body protein, but was simply being deposited as such. Recently, Terroine and Mahler-Mendler (1927) found in young pigs that gelatin and ammonium citrate increased equally the amount of protein retention already present on the basal ration; while on a diet low in protein, they each decreased the protein loss to the same extent. Similarly, when the basal diet already permitted maximum nitrogen retention, part of the casein contained in the diet could be replaced by gelatin or ammonium citrate, without reducing the nitrogen retention. It is hardly within my present scope to review again the overwhelming

evidence that indicates the relatively unsatisfactory function of certain proteins in nutrition, due to their incompleteness. Papers such as the foregoing, however, raise the question as to whether synthesis of protein from ammonium salts, if it should occur, would not produce, at best, incomplete proteins of the gelatin type.

V. The Importance of Carbohydrate in Relation to Protein Synthesis. I pass over the evidence of Kostytschew and Brilliant (1923), Neuberg and Kobel (1925, 1926), Borsook and Wasteneys (1925), and others, proving beyond doubt that not only can amino acids and sugar react to form stable compounds, in vitro, but that they can do so spontaneously and under conditions not far removed from the physiologic (this conception dates back to Lüthje). These experiments, as well as those showing the existence of protein sugar compounds in the blood and tissues, have only a contribution by analogy to make to our present problem.

a. The protein-sparing action of carbohydrate. Assuming for the moment the possibility of protein synthesis, what is the relation of carbohydrate to the process? In this connection we should examine the behavior of carbohydrate in sparing endogenous protein metabolism. That carbohydrate has this power has long been known, and it was shown by Voit and Bischoff as early as 1860 that carbohydrate spares protein better than does fat. The supporting evidence is voluminous, and the observation may be accepted as fact. For a long time, however, it was assumed that this could be explained on dynamogenetic grounds. Kayser (1893) and more particularly Landergren (1903) attacked this conception. The latter found that in nitrogen hunger in man, the capacity of carbohydrate to spare protein was twice as great as an isodynamic quantity of fat. On the basis of the fact that when the amount of protein in the diet was increased above the minimum breakdown, the discrepancy between the sparing action of carbohydrate tended to disappear. Landergren formulated the hypothesis that a certain amount of carbohydrate was essential for the cell protoplasm, that a certain breakdown of protein above the "minimum" was necessary to supply this carbohydrate, when it was lacking from the diet, and that only carbohydrate and not fat could "spare" this particular protein breakdown.

Cathcart (1909), however, obtaining results similar to those of Landergren, and observing in addition the fact that the output of creatine falls below the starvation level on a carbohydrate diet, but rises above it on a fat diet, went a step further. Supporting the conception of Paton (1905), he stated: "The hypothesis is put forward that the carbohydrates are absolutely essential for endocellular synthetic processes with protein metabolism."

Murlin (1907) performed experiments on men to test the cause of the (inadequate) protein sparing power of gelatin. His observations showed that gelatin spared protein far more than did the theoretical amount of sugar obtainable from gelatin (60 per cent). He therefore concluded that the protein sparing power of gelatin was not due to its contained carbohydrate. Lusk (1929) in commenting on the experiments of Murlin, (in which the removal of gelatin from a diet which otherwise contained one-third of the fasting requirements resulted in an increased nitrogen deficit in the presence of abundant carbohydrate), states: "Hence Landergren's interpretation that the rise in nitrogen elimination, which takes place on changing from a pure carbohydrate to a pure fat diet, is due to the body's absolute requirement for carbohydrate and that it obtains this by increasing its protein metabolism is scarcely tenable." However, it seems to me that although Murlin's experiment proves that the absolute requirement of the body for protein is due in part to a need which cannot be satisfied by carbohydrate (the "minimum nitrogen requirement" of Landergren) but which gelatin can to some extent supply (which is Murlin's contention), it does not prove that some of the protein requirement may not be due to the cellular need for sugar. In Murlin's experiment, for example, the protein deficit may have increased still more if carbohydrate were entirely removed from the diets containing insufficient protein.

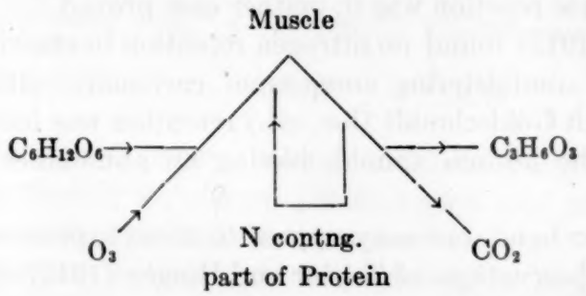
Murlin also found that carbohydrate failed to spare protein when given in small amounts, and that its protein sparing ability increased as the amount given was augmented, from which he deduced that only carbohydrate in excess of the metabolic requirements for energy is significant for protein sparing. It followed that the sparing action of carbohydrate was due to some other action than substitution for combustion. Rubner (1908), however, believed that the difference between the action of the readily soluble and utilizable carbohydrates and fats could still be explained on purely dynamic grounds.

Ringer (1912) found in phlorhizinized dogs that the amount of protein spared by "catabolized" glucose was considerably more than the latter's dynamic equivalent of protein (in one case 5.5 grams spared 81 grams). These experiments support Landergren. Ringer, though he speaks of "catabolized carbohydrate," assumes that the sugar is not oxidized. Of this I shall have more to say in another section. For the present, it will suffice to say that Ringer only uses the argument to show that even if the sugar were oxidized, it could still not account for the protein spared on dynamo-genetic grounds. Similarly, Ringer and Frankel

(1914) found that acetaldehyde and propylaldehyde also spared protein in phlorhizin diabetes. Assuming these to be products of the metabolism of carbohydrate, it again postulates a function for carbohydrate apart from its caloric contribution. Kocher (1916) made the observation in normal dogs that lactic acid spared protein as well as did cane sugar; pyruvic acid acted similarly, though not so well.

The general tenor of the above cited experiments on protein sparing indicates a function of sugar and its split products apart from their function in supplying energy directly, by oxidation. I believe this evidence may be accepted, yet it has only an inferential value in the present discussion, for while it *may* be used to postulate an association of carbohydrate with protein synthesis, it offers *no proof* that such is the case.

b. The theory of carbohydrate action in protein synthesis. I will not here go into the evidence that protein synthesis in plants may necessitate the presence of carbohydrate; nor into that which indicates a combination of sugars with simpler nitrogenous compounds in vitro. (References to literature relating to this may be found in Cathcart's *The Physiology of Protein Metabolism*, London, 1926.) In the animal, the conception that the products of carbohydrate metabolism are involved in amino acid synthesis can really be dated from Knoop's hypothesis of the reversal of oxidative deaminization. It is true that in 1905 Paton conceived that during muscular activity a marked increase in nitrogen loss was prevented by a synthesis of protein, as follows:



But he offered no definite evidence for this. Cathcart (1909), as has been seen, upheld this notion. Knoop's conception, supported by his experimental evidence and that of Embden and his co-workers (loc. cit.), embraced the idea that the keto acids resulting from carbohydrate metabolism could be converted, via a hydroxy amino acid, to an amino acid in the presence of ammonia, thus:

$$R\cdot CO\cdot COOH + NH_3 \rightarrow R\cdot COH\ NH_2\cdot COOH \rightarrow R\cdot CHNH_2\cdot COOH$$

The experiments of Fellner (loc. cit.) in Embden's laboratory further confirmed the intervention of carbohydrate in the synthetic process, for she found that on perfusing the glycogen-rich liver with blood to which ammonium chloride was added, alanin could be recovered in the perfusate, but not when the glycogen-poor liver was perfused.

Dakin and Dudley (1913, 1, 2) postulated reversible series of reactions between carbohydrate and amino acids, as follows:

$$\begin{array}{c} C_6H_{10}O_5 \\ \downarrow\uparrow \\ C_6H_{12}O_6 \\ \downarrow\uparrow \\ CH_3 \cdot CHOH \cdot COOH \rightleftharpoons CH_3 \cdot CO \cdot CHO \rightleftharpoons CH_3\,CHNH_2 \cdot COOH. \end{array}$$

They regard the theory of the formation of ketonic aldehydes from amino acids as indicating "a probable mode of synthesis of amino acids in living cells from nitrogen-free aldehydes and ammonia." But they admit that while the reversibility of the reactions between glycogen, glucose, methyl glyoxal, and lactic acid has been demonstrated, the direct formation of alanine from methyl glyoxal has not. They quote Neuberg and Langstein (1903) and Embden and Kraus (1912) in regard to the interconvertibility of lactic acid and alanine in the animal body. But though Neuberg and Langstein, on giving alanine to rabbits found an increase of liver glycogen, and though Embden and Kraus, by liver perfusion, obtained evidence for the conversion of alanine to lactic acid, the reverse reaction was in neither case proved.

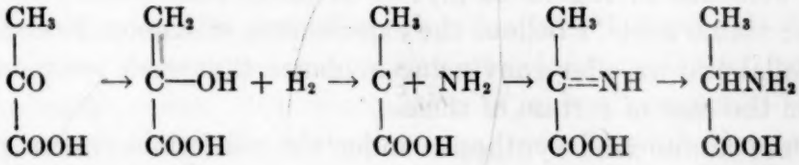
Underhill (1913) found no nitrogen retention in starving phlorhizinized dogs on administering ammonium carbonate; although in collaboration with Goldschmidt (loc. cit.) retention was found to occur in the case of the normal animal, having an abundance of oxidizable carbohydrate.

On the other hand, one may oppose to these experiments and those of Grafe the observations of Taylor and Ringer (1913) who found that on feeding ammonium carbonate to starving dogs, where the carbohydrate supply was at a minimum, there was a considerable retention of nitrogen which was not later eliminated. In one experiment, "the nitrogen of the ammonia affected the nitrogen balance almost to the same extent as the feeding of meat containing the same amount of nitrogen." They state further: "These experiments prove that ammonia can be retained in the absence of carbohydrates, and Grafe's theory can therefore be discarded." But their argument involves an accept-

ance of the theory of reversible oxidative deaminization (they regard this reversibility as a function of the law of mass action), and while the products of fat metabolism might be considered as playing a part in such a process, so might also the split-products of carbohydrate.

On weighing the evidence, the exclusive use of carbohydrate in supplying the non-nitrogenous portion of the molecule in protein synthesis must be regarded as not proved, and we revert to the original question of whether or not protein synthesis actually occurs in the animal body.

The latest word on the whole subject belongs, as though by right, to Knoop. With Blanco (1925) he observed the formation and secretion of acetylated amino compounds in the animal body. The amount of acetylated amino acid was increased after giving pyruvic acid, but not with acetic or butyric acid. With Oesterlin (1925) Knoop made use of the newer laboratory methods in the production of naturally occurring amino acids. He assumed the first phase of oxidative deaminization to be a splitting off of two hydrogens in Wieland's sense, followed by conversion (through an amino acid) to a keto acid. In the presence of nascent hydrogen and such catalyzers as platinum or palladium and at room temperature and normal pressure, these authors greatly increased the yields of amino acids which had been obtainable in the earlier experiments of Knoop and his collaborators. The reaction may be written as follows:



In a beautifully written paper—in length it is hardly more than a note, but its contents dignify it—Knoop (1926) argues that the conditions of his *in vitro* experiments and of the animal body are similar. He regards these reactions as important in the animal body, and conceives that when the body lacks one of the foodstuffs, it can build it up. Protein, he feels, is no exception.

VI. Summary. The treatment given to the preceding section may appear frequently not to have been germane to the question with which we are really concerned, namely, whether carbohydrate or fat or their split-products can be converted to protein in the animal body. But it will be obvious that such a conversion is conditional upon the problem of whether synthesis of protein (apart from the linking together of

amino acids) can occur at all. This in turn is dependent upon still other evidence. The logical frame-work would therefore be somewhat as follows:

1. Can protein synthesis occur in the animal body from nitrogenous compounds simpler than the amino acids?

But this question is dependent primarily upon experiments in which such compounds are administered to animals, and in which the effect upon retention is studied. We must therefore first ask the question:

a. Is nitrogen retained when these substances are administered?

In herbivora, the answer to this question appears to be in the affirmative, though there is considerable evidence to indicate that the retention is, in part at least, the result of bacterial action in the gastro-intestinal tract. In carnivora, the answer is more difficult. In my opinion, the weight of evidence is in favor of the view that such a retention occurs. This permits us to pass to the next question.

b. Does nitrogen retention under the conditions mean amino acid synthesis?

This point, I believe, is not proved, though on the whole, the evidence induces a presumption in favor of it. The possibility, at least, has not been disproved.

c. Does other evidence prove that individual amino acids may be synthesized in the body?

The evidence in regard to glycine is fairly conclusive. In regard to other amino acids, I believe the experiments of Knoop, Embden and their collaborators offer convincing evidence that such synthesis may occur in the case of certain of them.

d. Does amino acid synthesis, under the conditions, mean protein synthesis?

I believe this is not proved, but is possible. Certainly the formation of certain amino acids from keto acids and aldehydes cannot be regarded as proof of protein synthesis. Yet "aminization" in itself may be quite easy for the body to accomplish, provided the right sort of non-nitrogenous material is present. As Neubauer (1928) points out, it may be in the building of the proper kind of "carbon skeleton" of certain of the amino acids that the organism fails. One may even assume that the amino acids capable of being synthesized can be linked to form polypeptids and even incomplete proteins, comparable, for example, to gelatin. If one then assumed the retention of certain "irreplaceable" amino acids during endogenous or exogenous protein catabolism, and the linkage of these amino acids with those formed as above, the chain

of protein synthesis would be complete. Needless to say, this structure is largely hypothetical, and can only be admitted as a possibility, though a tempting one.

2. Assuming the possibility of protein synthesis as above, does it involve carbohydrate?

On the evidence of the experiments on nitrogen retention with and without carbohydrate, this is not proved. The theoretical evidence from *in vitro* experiments, as well as liver perfusion experiments, indicates only that if synthesis occurs, keto acids and aldehydes play a part in the process. But these non-nitrogenous substances may arise from both carbohydrate and fat metabolism.

To summarize: Metabolic products of carbohydrate and fatty acid metabolism may, and probably do, enter into the synthesis of certain amino acids from simpler nitrogenous compounds. That such a synthesis of amino acids indicates a normal synthesis of protein in the animal body is not proved, though the possibility exists. There is practically no present evidence to indicate that such protein synthesis, if it occurs, is of very great importance in nutrition.

THE CONVERSION OF PROTEIN TO FAT. Theoretically, there is no reason to doubt the possibility of the conversion of protein into fat. As we shall see later, there is hardly any question that protein can be converted to carbohydrate, and equally little that carbohydrate can be converted to fat. Moreover, there is ample evidence that fatty acid radicals, at least, arise in the course of amino acid catabolism. Since these processes can be carried out by the animal organism, it would seem to follow as a matter of course that the formation of fat from protein may also occur. But recognition of this theoretical likelihood implies neither that it is a normal concomitant of metabolism, nor that it occurs in unusual circumstances, such as high protein feeding or pathological states. The proof must lie in experiment, and it is not generally admitted at the present time that experiment has offered fully convincing proof.

Liebig, chiefly on the fact that he was able to find fatty acids upon treating a protein such as casein with caustic potash, was of the opinion that fat could come from protein. But in his case, as well as in that of the other investigators of the period—both for and against the hypothesis—the evidence was too vague to merit much more than a historic interest.

The major interest in the problem during the early stages of its investigation arose from the classical dicta of Rokitansky and Virchow. The

latter first in 1843 stated his view that a pathological amount of fat in an organ was due either to "infiltration" from elsewhere, or to "degeneration." "Degeneration" he assumed to be due to breakdown of the tissue protoplasm, with the formation of fat from the protoplasmic protein. This statement, bearing the weight of Virchow's authority, and supported later by apparent physiological proof on the part of Voit and his pupils, resulted in general acquiescence, and it was not until the last decade of the century that the theory came in for serious criticism. But before discussing the development of the problem along these lines, we may briefly examine the occurrence, if any, of the conversion in the simpler forms of life.

I. In Lower Organisms. This need not detain us long, for the bearing upon the question, except by analogy, is not great.

a. By bacteria and yeast. Naegeli and Loew (1878) found that the fat content of growing yeast cells increased, and that the increase was proportionate to the rate of growth. As the fat content was found to be chiefly intracellular (as shown by digestion) Naegeli contended that it must come partly from protein, though he admitted that carbohydrate might also be a source of the fat. Maclean (1922) repeated these experiments, and confirmed the increase of fat as observed by Naegeli, but found that the loss of cell protein that occurred could not account for all of the fat formed, and regarded its source as the carbohydrate material stored in the cell. Fat may arise from protein in the growing yeast, but there is no proof that it does so.

A similar doubt occurs in the case of ripening cheese, often cited in the earlier days as an example of the conversion of protein to fat. This dates from the experiments of Blondeau (1869) who found an increase of fat from a content of less than two per cent in the fresh cheese to as much as thirty-two per cent at the end of a year of ageing. The casein, in the meanwhile, had dropped from 85 per cent to 40 per cent. Such huge changes were not observed by anyone else, though Jacobsthal (1893) found that a ripening cheese increased its fat content a hundred fold in fourteen days. During the years between these observations, and even afterwards, a voluminous literature arose on the subject. Sometimes a small increase in fat was observed, sometimes a decrease.

It will suffice to quote the experiments of Kondo (1914), in which the fat determinations in such a research were carried out for the first time by an adequate method (Kumagawa and Suto). Kondo found that the fat content of ripening Cheddar cheese actually decreased as much as twelve per cent.

The ripening of cheese cannot be used as an example of the formation of fat from protein.

Nevertheless it appears that bacteria are in certain cases, at least, able to convert protein to fat. An example of this may be cited in the experiments of Beebe and Buxton (1905) who noted that bacillus pyocyaneus, grown on sugar-free meat broth and Witte's peptone, formed a pellicle composed of fat. Also Walker and Ainley (1922) observed that bacterial cultures grown in a carbohydrate-free medium contained more fat than was present in the medium, the inference being that fat was formed from protein.

b. By developing eggs. The earliest experiments on this subject were performed by Hofmann (1872), who hatched blow-fly eggs into their larvae and found that the larvae contained as much as ten times more fat than did the eggs or the blood on which they were nourished. No account was taken of the sugar in the blood (although it is true this would not have accounted for all of the fat observed). Pflüger criticized this work on the ground that bacteria in the unsterile material might have accounted for any conversion of protein to fat that occurred. Tangl and Farkas (1904), also using antiquated methods of fat determination, noted that the fat content of trout eggs increased during development. Weinland (1908) observed that the larvae of the blow-fly could form higher fatty acids from peptone or amino acid mixtures, though the eggs could not. A pulp of these larvae had the same ability. This was developed in a series of long papers. Gortner (1914), working with salamander eggs, noted an increase in ether soluble substances in the developing eggs. There was no change in the nitrogen content, (noted as well by Tangl and Farkas) and Gortner concluded that a carbohydrate radical combined with protein was partly converted to fat. McClendon (1915), also using salamander eggs, observed a small increase (eight per cent) in the fatty acids during development, and as the eggs gave no Molisch reaction, concluded that the fat came from vitellin. Brook trout eggs showed a similar increase in fat. The experiments of Nishikata (1922) indicated that developing fly maggots could increase their neutral fat content (as distinguished from fatty acids), on a nutrient medium containing no carbohydrate and not enough fat to account for the increase. The action of bacteria, it must be confessed, is not entirely ruled out in any of these experiments, but it would seem that developing eggs and larvae, as well as bacteria, can achieve the production of fat from protein.

II. In Pathological Conditions. I have already referred to the interest

in the conversion stimulated by the views of Virchow on fatty degeneration. In fact the early concern with the subject was largely related to its pathological implications. Much of the literature has been devoted to this phase of the matter, and particularly to the relation of the conversion of protein to fat during the production of adipocere, and during phosphorus and phlorhizin poisoning.

Adipocere. At one time there was considerable preoccupation with the formation of adipocere (the literature on the subject is given by C. Voit (1881) and by Rosenfeld (1902)). It seems hardly worth while to enter into this now, for in the first place it is highly questionable whether adipocere represents a conversion of protein to fat at all; in the second place, even if it does, there is practically no experimental work proving that such a conversion is not caused by bacteria. A discussion of the phenomenon, therefore, would at best simply bring us back to the ability of bacteria to form fat from protein, which is admitted.

Fatty degeneration. The certainty that the fat arising in large quantities during certain pathological conditions derived from protein would be strong presumptive evidence in favor of a similar occurrence in the normal, even though it were not proof. Rokitansky, who first discovered the fatty liver and kidney in phosphorus poisoning, did not attempt to discover the origin of the fat, nor did Virchow, when he elaborated his theory. Lebedeff (1883) first attacked this problem, and while he demonstrated a deposition of fat in various organs, he did not rule out the possibility of fat synthesis, for he did not determine the total fat, or the amount in the usual fat depots. Leo (1885) poisoned guinea pigs, rats and frogs with phosphorus, and starved them until they were killed. The animals were compared with normal animals also starved as to fat content. The phosphorized rats contained less fat than the unpoisoned ones, but in the case of the guinea pigs and frogs the animals treated with phosphorus contained considerably more fat than the controls. This, according to Leo, could only have been due either to 1, failure of oxidation of the fat, or 2, fat synthesis. Ruling out the former somewhat arbitrarily, he came to the conclusion that in the phosphorized animals fat was synthesized from protein. The "ethereal extract" in these experiments was called fat, and in other ways, such as the choice of controls, the experiments were faulty, and were justly criticized by Pflüger. They cannot be said to have been in any sense conclusive. The same applies to the experiments of Polimanti (1898) who observed in control animals which had been starved 4.47 per cent of fat, while animals of the same sex (since male frogs have

less fat than female frogs), which had been poisoned with phosphorus and killed after the same period of starvation, contained 5.51 per cent of fat. He concluded that a production of fat from protein had taken place. But Pflüger again pointed out that the frogs might have had enough glycogen left to account for the fat, assuming that a greater conversion of carbohydrate to fat occurred in the phosphorus animals. The experiments of Woltke (1901), in which the iodine number of the fat in phosphorus poisoned dogs fed with linseed oil was compared with that of control dogs, purported to show the absence of fat migration in the phosphorized animals. The I_2 number of the control dogs' fat was 100–116, while that of the phosphorized dogs was 63–65. However, even if these experiments indicated that the fat found in the poisoned animals was not linseed oil, they did not rule out a migration of the dogs' own fat to the liver and viscera. Wells (1905) attempted a similar experiment in rabbits, injecting iodipin subcutaneously, and could find no certain iodine reaction in the fatty liver and kidneys of the phosphorus rabbits. But again, fat migration to these organs from elsewhere in the body was not ruled out.

On the whole, the positive evidence in favor of Virchow's theory has in itself been dubious, but in addition, there is experimental evidence testifying to its unsoundness. Athanasiu (1899) found that the total body fat underwent no change under the influence of phosphorus, but that there was regularly an increase in liver fat. He concluded, therefore, that the liver fat had migrated from elsewhere in the body.

In a series of communications, Rosenfeld (1893, 1895, 1, 2; 1897, 1899) offered what is probably the most conclusive evidence in favor of fat "infiltration," as opposed to fatty "degeneration." His first paper was preliminary, and led to only a presumption in favor of the migration of fat into diseased organs. In his second (1895, 1) his evidence was more concrete. He performed the experiment of first starving normal and phosphorus poisoned dogs; then feeding them with mutton fat, then starving them for seven days. Under these conditions the normal dogs showed mutton fat under the skin, in the omentum, etc. The liver, however, during the second starvation period, gave up all but a constant ten per cent of fat, which was dog fat and was considered by Rosenfeld an integral part of the structure. But in the phlorhizinized animal, the dried liver contained 55 to 65 per cent of fat, of which all but ten per cent was mutton fat. The conclusion was inescapable that the "pathological" liver fat had actually migrated from fat depots elsewhere in the body.

In his paper of 1897, Rosenfeld reported a repetition of the above experiments, using in this case animals poisoned with phosphorus, and obtaining similar results. Moreover, he observed that starved fowls do not develop fatty livers in phosphorus poisoning, while well nourished fowls do. On the basis of the above observations, he denied that the "pathological" fat of "fatty degeneration" came from protein. Going on a step further, after reviewing the work of others, he stated that all fat in the body comes either from fat or carbohydrate in the diet. These opinions he elaborated in two articles in the Ergebnisse der Physiologie (1902, 1903).

The views of Rosenfeld in regard to fat migration were supported by Ray, McDermott and Lusk (1899); by Kraus and Sommer (1902); by Leick and Winckler (1902); and by Shibata (1911). Lusk advanced and still supports (1928) the theory that fat is attracted in larger quantities than can be useful to any cell which is sugar-hungry, or in which carbohydrate is not oxidized.

TABLE 1
(Lindemann)

	"DEGENERATION" FAT	NORMAL HEART FAT	"INFILTRATION"* FAT
Acid number	18.35	7.3	3.76
Saponification number	257.4	202.3	201.8
Iodine number	108.55	61.1	70.8
Volatile acids	23.9	2.0	0.93

* The "infiltration" fat was an average of kidney, subcutaneous, and pericardial fat, similar to that found by other observers for body fat in general.

Reviewing the experimental evidence, it seems clear that the formation of fat from protein in such pathological conditions as phosphorus and phlorhizin poisoning is not proved, and that on the contrary, the weight of testimony is in favor of the idea that the "degeneration" fat is actually a deposit which has migrated from other parts of the body.

So far as I know, only one experiment similar to the above has been performed on human tissue, and this is against the migration theory. Lindemann (1899, 1) determined the acid number, saponification number, iodine number, and content of "steamvolatile" acids in normal and "degenerated" hearts. The individuals had either suffered from cardiac disease or were "normal" (dead from suicide, etc.). Table 1 illustrates the results.

Lindemann assumed that the infiltration fat would be the type of fat that would migrate, if migration occurs. Actually, the "degeneration" fat differed widely from both normal heart fat and general body fat. The conclusion was that there was no migration, and the inference was that the fat was formed from protein. However, it can hardly be admitted that the latter is proved, though it appears to offer good evidence against fat migration in this particular case. Unsupported, it is not sufficient to invalidate the general conclusion that pathological fat does not arise from protein.

In another paper, Lindemann (1899, 2) showed that in a skin preserved aseptically there was a formation of fat, of which the source may have been protein.

I may here mention the experiments of Berg and Falk (1924). These investigators found fat forming in the muscle fibers of frogs during the second half of the hibernating season—and only in those fibers which gave the ninhydrin reaction. They concluded that fat was formed from protein disintegration.

Experiments with isolated tissues. Experiments in which isolated tissues have been preserved for long periods of time have furnished very little support for the view that protein can be converted to fat. Siegert (1900) autolyzed sterile dogs' liver and obtained no increase in the percentage of ether-extract or of higher fatty acids. The possibility existed that fat destruction might have gone hand in hand with fat synthesis, but it seemed to the investigator that this would hardly occur. Hildesheim and Leathes (1904), on the other hand, did obtain an increase of from fifteen to thirty-three per cent on incubating rabbit livers. They added antiseptics, but the material was not sterile. They claimed that the results were not due to the organisms present, however, for when broth cultures of the bacteria were added to samples of boiled liver on incubation, there resulted no greater yield of fatty acids than when boiled broth cultures were added. The fact that the method of fat determination involved considerable error, as well as the fact that glycogen might have been a source of fat, made any conclusions that might have been drawn from these experiments as to production of fat from protein, of doubtful value. The authors, indeed, do not make any such postulate. They observed incidentally that the addition of glycogen increased their fatty acid yield. Saxl (1908) and Ohta (1910) were unable to observe any fat increase. The latter incubated horse liver for one hundred days, and using the new method of Kumagawa and Suto, determined the content of higher fatty acids before and after

autolysis. Recently, Terroine and Belin (1926) incubated sterile dog blood for a year and were unable to observe any increase in fatty acids and unsaponifiable substances. Leo and his collaborators (1913) performed experiments in which dog and rabbit livers, removed from the body aseptically, were incubated with and without phosphorus. An increased fat production was observed in some cases with phosphorus, but the results on the whole were negative as showing a direct effect of phosphorus on the liver. Moreover, the slight increases of fat might easily have come from glycogen.

The few experiments on isolated perfused tissues are equally negative. Cesaris-Demel (1909) perfused Langendorff-isolated hearts with Ringer-Locke's solution, the hearts being poisoned with arsenic, phlorhizin and diphtheria toxin, and found histological evidence of fatty degeneration. Hence he assumed that the fat came from within the cells themselves, and originated in the cell protein. But granting that this type of experiment rules out fat migration, it certainly does not prove the conversion of protein to fat.

Gross and Vorpahl (1914, 1, 2) perfused rabbit kidneys for twenty-four to forty-eight hours and found an increase in the cortical fat, both microscopically and chemically. Underhill and Hendrix (1915) repeated these experiments, and obtained the same results, but ascribed the increase to mechanical contamination on removing the cortex, for the kidney becomes edematous and friable on perfusion for this length of time. They obtained the same increase of fat in a kidney simply suspended in warm Ringer's solution for twenty-four hours, yet the cells must have ceased functioning very rapidly, hence even if a synthesis of fat were taking place, it could not have been a function of the living organ.

Thus the experiments on isolated tissues, both incubated and perfused, offer no support for the view that protein can be converted to fat.

III. Feeding Foreign Fats. Most of the experiments of this nature have been carried out on animals poisoned with phosphorus or phlorhizin. I have already outlined the trend of these observations, which indicate that a migration of the foreign fat was taking place to the diseased tissue. In a few other cases, foreign fats have been fed to normal animals, as for example in the experiments of Radziejewski (1872), who gave rape seed oil to starving dogs and was unable to find the erucic acid peculiar to the rape oil in the copious deposits of body fat. Ssubotin (1870) gave to dogs that had been starved a long time large quantities of meat and fat. In some cases, he gave palmoil, con-

taining no stearin, and found large amounts of stearin in the fat deposited as a result of the feeding; in other cases, he fed meat and a soap containing no oleic acid, yet in the deposit fat there was the usual percentage of oleic acid. Ssubotin therefore concluded that the fat laid down in the body must have come from protein.

But Radziejewski's experiments were repeated by Munk (1884) who was able to demonstrate the erucic acid that the former investigator failed to obtain. Munk also created a "mutton-fat" dog, as Rosenfeld and others did later. Moreover, the experiments of Radziejewski and Ssubotin do not in any case disprove that the fat in the body came from fat, for even if ingested foreign fats are often deposited as such, it does not follow that they must always be. During digestion and resynthesis, there is no reason why they should not be rebuilt into the animals' own fat.

In this connection the results of Abderhalden and Brahm (1910) are of interest. They gave mutton fat or rape seed oil to dogs that had starved for two weeks to a month. On sacrificing the animals, they found that the deposit fat after extraction with ether, resembled mutton fat or rape oil, as the case might be. But upon digesting the extract with gastric juices or dilute HCl, thus presumably breaking down the cell wall and obtaining the "true cell fat," the latter was found to give reactions quite different from that of the fat fed, and the same no matter which fat was ingested.

IV. The Experiments on Protein Feeding. While the researches upon pathological conditions and upon the feeding of foreign fats, as well as those concerned with isolated tissues, offer no ground for believing that the conversion to fat occurs, they do not, on the other hand, prove that it may not occur. The only real evidence that we have to indicate the probability of such an occurrence lies in the experiments in which large quantities of protein have been fed to normal animals. Work had been done by Kemmerich (1866) and by Ssubotin (loc. cit.), which seemed to indicate that the milk of animals fed with protein contained fat that originated from this protein. However, this rested on shaky foundations, and Rosenfeld (1897) was able to show mutton tallow in the milk of his "mutton-fat" dogs. Some slight evidence might be adduced from the literature indicating the probable production of fat from protein feeding as shown by gain in weight, but this, too, remains equivocal (cf. the experiments of Osborne and Mendel (1924)). The experiments of Maignon (1918, 1919) and of Maignon and Jung (1924) showing an increased liver fat on feeding white rats with casein or fibrin, are open

to the old objection that the liver fat may have migrated from other fat depots in the body.

We must turn chiefly, therefore, to investigations in which the carbon and nitrogen balance, the respiratory quotient, and the heat production have been used as criteria.

a. Evidence from the C and N balance. Voit, alone and with his collaborator, Pettenkofer (1862, 1869, 1870, 1871) performed experiments upon normal dogs, feeding them with very large amounts of lean meat, and noting the balance of carbon and nitrogen in the ingesta and excreta. When the amount of meat administered was large enough there was always noted a retention of carbon that was not accompanied by an appreciable retention of nitrogen.

In a typical experiment, Pettenkofer and Voit (1871) found that while there was practically nitrogen equilibrium, there was a carbon retention of 42.1 grams. If this carbon had been in the form of glycogen, it would have amounted to 151 grams, an amount which Voit considered impossible. As fat it represented a deposit of 56 grams. In discussing another type of experiment indicating a similar phenomenon (Pettenkofer and Voit, loc. cit.), the authors state: "In smaller amounts of meat the body still loses both protein and fat; with less loss as the amounts of meat given are increased until at 1500 grams the 'flesh' and fat content of the body remains the same. On giving more meat, the nitrogen is excreted but carbon is retained." In one experiment a dog received a diet of 1500 to 2000 grams of meat daily for eight days, and during this time laid down carbon corresponding to 1014 grams of fat. This would correspond to 1940 grams of sugar, and there is not that much sugar or glycogen in the body of a 30 kilo dog.

Incidentally they also noted a closer relation between the O_2 consumed and that calculated on assuming fat rather than carbohydrate as the substance deposited.

Voit (1881) in a critical résumé of the question, including a survey of the existing literature, states: "Since there is no other substance in which so large an amount of carbon can be deposited as fat, fat must have been formed from protein."

I have discussed these experiments in more detail, perhaps, than they warrant, but emanating, as they did, from the hands and brain of a master of metabolic research, they exercised an enormous influence upon the thought of the day, and in the minds of most physiologists settled the question until 1892, when the equally renowned Pflüger attacked both the facts and the conclusions therefrom with rather more than his customary vigor.

Rubner, Voit's own pupil, had in 1885 introduced one correction into the latter's calculations. Voit, without actually determining it, had assumed that the C/N ratio of lean meat was 3.68/1. Rubner found lean meat to have 50.46 per cent C and 15.4 per cent nitrogen, a C/N of 3.28/1. Pflüger (1892, 12) using this as the basis of the assault, recalculated Voit's experiments, using, in fact, a C/N rather less than that of Rubner, and obtained in his own laboratory (3.22/1). In one experiment, a retention of 41.9 grams of carbon was shown to be practically annulled by a correction of 39.6 grams. In another of Voit's experiments an assumed retention of 56.7 grams of fat became only 3.9 grams, which Pflüger regarded as within the limit of error.

There was no doubt that Pflüger was right in his criticism, and that Jove had nodded. And in 1894 Kumugawa and Kaneda performed a research on dogs that tended to confirm Pflüger's view. These investigators starved two young bitches from the same litter for twenty-four days. They then sacrificed one and determined the fat content. The other they fed for 49 days with the leanest possible horse meat (which was analyzed) and then determined the fat content of the animal. Calculating from the fat and glycogen in the meat, and the amount of fat laid down by the dog (who received 1200 gm. meat per day for the last twenty-nine days), they concluded that even on this high protein diet all of the deposited fat could be accounted for by the fat and glycogen in the food.

But in 1892, Erwin Voit published data that supported the conclusions of his brother. Admitting the errors in the older work, he performed experiments of which the following is an example. A 23 kilo dog received 1500 grams of washed meat daily. During the last two days there was a carbon retention of 89.7 grams. But there was also a retention of 18.0 grams of nitrogen; and using a C/N of 3.29/1, 59.1 grams of carbon would be accounted for as being possibly due to a deposit of protein. The remainder, 30.6 grams, could only have been deposited as fat, for it corresponded to 134 grams of glycogen, or 6.1 grams per kilo deposited in two days, and a dog, even after being well nourished for a long time, would not contain that amount.

But Pflüger, returning to the attack in 1897, stated that the true C/N for "washed" meat, which E. Voit used, was 3.1/1 (as obtained in Pflüger's laboratory). Recalculating, he found the 30.6 grams carbon retained according to Voit, was really 11.3 grams. Moreover, the C/N of the retained moiety was 3.7/1, which Pflüger said could be explained as the retention of material which was chiefly protein, plus a small

amount of certain amino acids, such as tyrosin, which is relatively poor in nitrogen and rich in carbon.

Again the criticism of Pflüger was sufficient to cast reasonable doubt upon the results, and as Cremer (1897) said, even a non-protein deposit might have been glycogen. This investigator reported experiments upon a cat of 3.7 kilos, which was first starved, then fed daily with 450 grams of lean meat for eight days. An average of eight experiments follows:

N in urine and feces	C excretion			Meat C Calc. from N excretion	C Deposit
	Urine	Feces	Resp.		
13.0 grams	7.5	1.4	25.4	41.6	7.3
		34.3			

The total carbon deposit of the period was 58 grams, corresponding to grams of glycogen. Actually, the animal on being killed showed a maximum of 35 grams of this substance.

Pflüger devoted the major portion of his 1897 paper to this research by Cremer. He criticized it on several grounds, such as the possible deaminization of protein and the excretion of urea from the amino acids plus the extractives, with the retention of the non-nitrogenous portion in the intestine, on the last days. He also suggested that the formation and deposit of "cat protein" from the beef fed might affect the ratio of C/N excreted. His chief criticism had to do with the C/N ratio of the deposit, which in Cremer's experiment was 7.1/1 (as some nitrogen was retained). Pflüger postulated that this might be due to the deposit of such a substance as tyrosin, which has a C/N of 7.7/1.

But his criticism, hitherto so destructive, seems here to be much weakened. While some of the factors to which he alluded may to a slight extent have influenced the results (as, for example, the neglected glycogen and fat content of the meat given), nevertheless their combined influence was negligible, and cannot be regarded as invalidating the author's conclusion. Cremer repeated his experiments in 1899 with results similar to those in his first series.

b. Evidence from the R. Q. and heat production. The fact has already been mentioned that Pettenkofer and Voit in their experiments found that the oxygen consumption of their animals corresponded more nearly to the theoretical if the deposit of fat were assumed rather than that of carbohydrate. But we have also seen that these experiments cannot be accepted at their face value. E. Voit (loc. cit.) also stated that the energy balance indicated a deposition of fat, but here also, the evidence is doubtful.

Kaufmann (1896, 1, 2, 3) reported data obtained on a dog by means of a respiration calorimeter. The animal chamber was large—2600 liters—and the dog rebreathed the same air, though Kaufmann maintained that the gaseous contents of the sealed chamber did not approach values that might alter the metabolism. Space forbids me to analyze his technique. Suffice it to say that it left much to be questioned, and it is rather difficult to see how he could obtain accurate results. However, upon giving meat, the amount of which is not stated, he found that both the respiratory exchange and the heat measured directly, were considerably less than would correspond to the total combustion of the metabolized protein, the latter being estimated from the nitrogen output. The average O_2 consumption was 7.82 L., with the production of 32 Cals., and he estimated that to burn the protein 9.64 L. O_2 would have been consumed and 45 Cals. produced. His calculations of the latter are based upon erroneous data; on recalculating, I find the theoretical figures to be 8.9 L. O_2 and 40 Cals. It appears therefore that if the results are sound a deposit actually occurred. Kaufmann attempts to prove that this deposit is fat, according to Gauthier's equations, or that part of the fat is converted to carbohydrate, according to the equations of Chauveau. However, the data upon which these calculations rest are, as I have indicated, so subject to error that it is difficult to say what the nature of the deposit actually was. For example, on recalculation, I have found deposited material to have an R. Q. of 0.55, which is clearly impossible. The case, therefore, remained unproved.

Lafon (1913) criticized the work of Kaufmann on several grounds, but chiefly because of the short duration of the experiments. Lafon himself gave a dog 1200 grams of meat daily, and for two periods of seven days each determined the gas exchange and the nitrogen excretion. He found a practically complete agreement between the respiratory exchange actually observed and that which would theoretically result from the combustion of protein corresponding to the urinary nitrogen excretion. Curiously enough, he regards this as showing the conversion of protein to carbohydrate, though he denies the conversion of protein to fat. The respiratory exchange that he observed after meat is open to some question, for the basal O_2 was 122 L. per day, while that after 1200 grams of meat was 206 L., a specific dynamic action of about 80 per cent for twenty-four hours! It is quite possible that Lafon would have obtained a deposit of carbon had it not been masked by the extra metabolism during muscular activity.

Williams, Riche and Lusk (1912) found a retention of carbon when

a dog of 13.5 kilos was fed 1200 grams of meat, but the authors concluded that this retention was in the form of sugar. The experiments of Lusk and his collaborators (1922) seemed to offer more convincing evidence than had previously been obtained from studies of the respiratory quotient and the heat production that under certain circumstances the production of fat from protein could occur. Upon giving large amounts of meat, these investigators found that there was much less carbon eliminated in the respiration than corresponded to the protein metabolism of the period as measured by the excretion of nitrogen in the urine, and that hence a deposit of carbon occurred. When 200 to 800 grams of meat were given, the respiratory quotient of the deposited material

TABLE 2

(Atkinson, Rapport and Lusk)

Fifth hour after 1,000 grams of meat. Urinary N = 1.44 grams per hour

	CO_2	O_2	CALORIES
	grams	*grams*	
Equivalent of 1.44 grams N	13.46	12.17	38.17
Found in respiration	10.10	8.72	
	3.36	3.45	

R.Q. of the deposit = 0.708

C retained = 0.92 gram = 1.2 grams fat = 11.32 Cals.
2.3 grams glucose = 8.63 Cals.

Calories if C was retained as fat = 38.17 − 11.32 = 26.85
Calories if C was retained as glucose = 38.17 − 8.63 = 29.54
Calories actually found (calculated from O_2) = 26.85
Calories actually found (directly measured) = 27.52

indicated that a mixture of carbohydrate and fat was being retained. Upon giving 1000 to 1300 grams of meat (as much as the animal would take), the R.Q. of the deposit tended to approach the theoretical value for fat during the early hours thereafter. Moreover, the formation of fat was confirmed by the agreement of the heat production as calculated on the assumption that fat was being laid down in the body. Table 2 gives a resumé of the method of calculation employed.

There can be little doubt that these results are suggestive. But do they constitute proof? Mitchell and Hamilton have recently (1929) criticized the results from several angles. In the first place they point out that the nitrogen excretion, over a short period of time in such an

experiment, cannot be accorded a high degree of accuracy in measuring the protein metabolism. This is admitted, yet if the other factors of the experiment are sound, the possible error resulting therefrom should not destroy the validity of the results. We might assume, for example, that the protein actually deaminized in the body corresponded, in the above experiment, to 2 grams N instead of 1.44 grams. If the non-nitrogenous portion of this excess (corresponding to 0.56 gram N) were oxidized, it would of course appear in the respiratory exchange. If not, it would tend to make the R.Q. of the deposit approach 0.80, its own quotient. But if this "deposit R.Q." still remained in the neighborhood of the theoretical value for fat, then it follows that fat was actually being laid down in the body. If sugar were the deposit material, the "deposit R.Q." would necessarily be above 0.80 irrespective of the error in the nitrogen determination. It can be calculated that if the protein metabolism of the period had corresponded to 2 grams N, the respiratory quotient of the deposited material would have been 0.74.

A second criticism involves the method of using the agreement between the theoretical and the found heat as a criterion of the conversion. The present reviewer believes this criticism to be, in a measure, justifiable and considers such an agreement as the weakest point of the argument. It is at best only confirmatory, being secondary in value to the respiratory quotient.

The third criticism by Mitchell and Hamilton has to do with the theory, implicit in the calculations, that protein alone is being oxidized during the experimental period. This criticism is important, if proper. Whether or not it is accepted depends on whether the oxidation of other substances—presumably carbohydrate—is regarded as probable in the presence of a plethora of the non-nitrogenous products of protein metabolism. One of the striking things about the administration of a moderate amount of protein to a dog is the fidelity with which the animal reproduces the theoretical R.Q. of protein. It is difficult to regard this as coincidence. In the experiment cited above, in which a large quantity of protein was given, the R.Q. was 0.84. This rise in quotient may have been due to the combustion of, let us say, 80 per cent protein and 20 per cent carbohydrate, or to an exclusive combustion of protein plus conversion to fat. It is hard to understand why, assuming that the animals' R.Q. of 0.80 or 0.81 on ingesting a smaller quantity of meat was due to the combustion of protein, the addition of more meat to the diet should have resulted in the burning of sugar.

The reviewer is unable to agree that the criticisms of Mitchell and

Hamilton warrant the conclusion that the investigation cited offers only presumptive evidence, rather than convincing proof, that the conversion takes place under the conditions. The chief difficulty with experiments of this nature is one not mentioned by Mitchell and Hamilton, namely, the accuracy with which the gaseous exchange must be obtained to render the conclusions valid. If the true respiratory quotient in the above experiment had been 0.80 instead of 0.84, the "deposit R.Q.," instead of being 0.71, would have been 0.82. That such a divergence was in fact beyond the limit of the experimental error was shown by the alcohol checks, but even were it admitted, the deposited material would still have to be considered as largely fat. Taking all of the facts into consideration——the occurrence of a non-protein carbon deposit, the rise of the R.Q., above the theoretical R.Q. of protein, the inherent improbablity of the oxidative metabolism being to any considerable

TABLE 3

Ingestion of increasing amounts of beef

DIET	TOTAL METABOLISM	PROTEIN METABOLISM (CALS. FROM URINARY N)	R.Q.	NON-PROTEIN R.Q.
200 grams beef	21.4	12.5	0.81	0.82
400 grams beef	22.8	19.5	0.82	0.91
600 grams beef	24.9	26.1	0.81	(0.84) Deposit
800 grams beef	26.3	34.0	0.83	(0.72) Deposit

extent non-protein in character, and finally, the thermodynamic agreement—all tending to the same conclusion, it seems inevitable that fat must have been formed from protein.

It is suggestive, moreover, that experiments by Weiss and Rapport (1924), undertaken for another purpose, duplicated the results mentioned above, as will be seen in table 3.

In these experiments there was again a tendency for the R.Q. of the deposited material to fall as the amount of protein ingested was increased.

It must be remembered, however, that even if this evidence is accepted as indicating the production of fat from protein, it does not furnish proof that this is a normal occurrence in the body. The conditions were exceptional in that large quantities of protein were given, and in addition to this, and partly because of this, the glycogen deposits were filled. In the later experiments of Atkinson, Rapport and Lusk, the conversion was facilitated by "charging the glycogen reservoirs," as Lusk expresses it, with a standard diet containing 70 grams of starch every evening.

As will be discussed in the ensuing section, the conversion of protein to fat does not appear to be less likely to occur than the conversion of carbohydrate to fat. Moreover, although the latter is at present accepted without question, while the former is not, even the difference in the facility of the conversion in the two instances may not be as great as is often thought.

V. The Mechanism of the Conversion. Theoretically, the possibility exists of a direct formation of fatty acids from amino acids, without carbohydrate as an intermediary stage. Such a conversion, for example, was indicated in the experiments of Neuberg and Rosenberg (1907) who showed that there was a formation of optically active valerianic and caproic acids in the metabolic breakdown of meat. Neuberg (1911) extended this by demonstrating that these acids were obtainable on the breaking down of leucine, and argued that this might be one of their sources in the protein molecule. One may assume that the lower even-C fatty acids may condense to form longer chains, with a final union with glycerol to form fat. The actual demonstration that this is a normal chemical process in the body, however, is lacking, and from the theoretical point of view, there is no reason for supposing that amino acids, if they are converted to fat, do not pass through a carbohydrate stage. On the other hand, it is not essential to postulate that an actual formation of hexose must have taken place before fat is formed, for the reactions may pass through simpler forms that are common to both amino acid and carbohydrate metabolism. If amino acids should pass either through hexose, or through compounds that are intermediary carbohydrate metabolic products, the mechanism becomes a problem, first, of the conversion of the amino acids to the above substances; second, of the conversion of carbohydrate or its split products to fat. Each of these is discussed in its proper place.

There is no valid reason, in the evidence that has been presented in this section, for believing that the conversion of protein to fat must be a direct process. For example, in Cremer's investigations the analysis of the animals after death showed that there was insufficient glycogen to account for the carbon deposit. Yet this is certainly no reason for postulating a direct conversion of protein to fat, for it is at least possible that glycogen or glucose was an intermediary step in the process. Moreover, in Cremer's experiments the muscle glycogen was very high (1.47 per cent) higher even than the amount found by E. Voit (see section on carbohydrate to fat) on administering large amounts of carbohydrate.

The same argument, it seems to me, applies to the experiments of

Atkinson, Rapport and Lusk, and of Weiss and Rapport. It is possible to suppose, of course, that when the glycogen stores are saturated, an alternative pathway is chosen which does not include the carbohydrate stage for the formation of fat. But it is equally possible that when the body has formed the maximum amount of glycogen from protein, any newly formed glycogen is in turn changed into fat. The experiments above cited throw no light on the matter. They can be interpreted in either way. Even if the conversion should go through carbohydrate, however, the ultimate origin of the fat would naturally be protein.

VI. Summary. None of the older evidence purporting to show the formation of fat from protein actually does so. This includes the oft-quoted work on adipocere, on fatty "degeneration" in its manifold forms, on the reaction of the body to foreign fats, on the formation of milk fat, as well as the earlier experiments on the carbon and nitrogen balance. The investigations dealing with autolyzed and perfused tissues have been equally sterile of results. Such valid evidence as exists comes from the material balance experiments of Cremer, and from the more recent investigations of the respiratory quotients and heat production following excessive protein ingestion. I believe that the data favor the view that, under the conditions, the conversion of protein to fat in the animal body actually occurs. Such a conversion may or may not go through a carbohydrate stage, though the former would appear at present the more likely.

Finally it should be emphasized that no evidence has been brought forward to indicate that the formation and deposition of fat from protein or amino acids (to be distinguished from the formation of lower fatty acid radicles in amino acid catabolism) is a normal and constantly occurring phenomenon in the animal body. Should it occur, it appears to do so only under unusual conditions of excessive protein ingestion.

THE CONVERSION OF PROTEIN TO CARBOHYDRATE. Claude Bernard, at the College de France, performed the first experiments purporting to show that sugar could originate from protein. His experiments were performed on normal animals, and the criterion of the conversion was the presence or absence of glycogen in the liver. In one investigation, Bernard fed dogs on meat exclusively for six to eight months, and found the normal amount of glycogen in the liver. He repeated this experiment with owls. Later on, he starved dogs for a week or more, then gave washed fibrin to the animals, and upon sacrificing them, found considerable quantities of liver glycogen, as was not the case when fat alone was given. In 1877 he summed up these experi-

ments in his "Leçons sur la Diabete." Adhering to his original doctrine, he recognized errors in his earlier work, particularly the fallacy of not knowing that starvation reduces the liver glycogen unequally in different animals. In this monograph he reported work he had done in 1858 and 1859. In these experiments, after the dogs had been starved from five to fourteen days, the abdomen was opened and a piece of liver was excised. Gelatin was then introduced into the stomach, another piece of liver excised after an interval of about eight hours, and on the following day the animals were killed and a third piece of liver was obtained. The three specimens were examined for glycogen, and the two later ones showed an increase in glycogen content. A positive result was also obtained with this technique when a dog was fed gelatin for a week, while another animal that was given lard for the same period showed no increase in the liver glycogen.

These experiments are easy enough to criticize—on the basis of the inaccuracy of the glycogen determinations, on the failure to rule out other sources for the glycogen, etc.,—yet they constitute the first scientific approach to the conversion with which we are dealing.

Bernard also performed certain experiments on fly-maggots, nourishing them on a protein medium such as egg albumen or meat, and found "much glycogen" in the larvae. This experiment was later repeated by Külz (1881), who criticized Bernard for not stating definite figures, and who, although he found some increase in the glycogen content of the larvae, was not prepared to admit the formation of glycogen from protein on the basis of such experiments.

Just as the early experiments of Pettenkofer and Voit on the conversion of protein to fat were accepted without sufficient critical analysis, so the work of Bernard on normal dogs was generally regarded in his time as conclusive proof of the conversion of protein to carbohydrate. His conclusions were, moreover, supported by the investigations of Wolffberg (1876), who observed much higher glycogen percentages in the liver and muscles of hens (up to 1.6 per cent in the former) after protein feeding, than when the birds had been starved. Experiments upon diabetic animals were also indicative of the conversion. It was not until Pflüger (1892) addressed himself to the task of destroying unproved conventions in regard to the conversion of proteins, that any doubt occurred as to the formation of sugar from them.

The evidence that has since accumulated leaves little doubt that such a conversion may occur. Most of the proof, however, is derived from experiments upon the various forms of diabetes, in which the formation

of sugar is comparatively easy to study, and in such data the question always arises, rightly or wrongly, whether the conditions are applicable to the normal animal. Assuming, for example, that in the diabetic state sugar can be shown to arise from protein, does it necessarily follow that this is an obligatory phase in the normal catabolism of the amino acids? What evidence, then, exists as to occurrence of the phenomenon in the non-diabetic animal?

I. Evidence for the Conversion from the Non-Diabetic Animal. a. Glycogen deposit and the blood sugar. In the discussion of the conversion of protein to fat, we have had occasion to mention the experiments of Cremer (1899) upon cats. In these experiments it will be recalled that there was a deposit of C in the body without a concomitant retention of N, and the conclusion was drawn that fat had been formed from protein. But in these experiments, as in others of a similar nature, it was assumed that carbohydrate could be formed as well, and the proof that fat had been deposited depended on the fact that a retention of C had taken place which the amount of body glycogen could not cover. For our present purpose, this is to some extent begging the question, though the probabilities are that a carbon retention represents either carbohydrate deposit, or fat deposit, or both. Actually, Cremer noted a maximum deposition of glycogen in the muscles (1.47 per cent) upon one-sided protein feeding, greater even than that observed by E. Voit (1892) in geese after starch ingestion. It would appear, therefore, that before fat could be deposited, there must have first been a maximal filling of glycogen depots. This conclusion was attacked by Pflüger, and by his pupil Schöndorff (1900, 1901), on much the same grounds as had called forth Pflüger's polemics against the conversion of protein to fat. Schöndorff, using frogs, could find no evidence of the deposition of glycogen on feeding them with casein. (At this time, the conception of carbohydrate complexes linked with the protein molecule was current, and Schöndorff, as well as other investigators, used casein because it was supposedly free from these linkages.)

Bendix (1901, 1902) obtained a definite deposit of glycogen on feeding casein to dogs whose glycogen store had been reduced to a minimum by hunger and muscular work.

Stookey (1903) gave various proteins to hens, and while on the whole his results were inconclusive, it appears from his data that the feeding of casein, at least, resulted in an increase in liver glycogen. In the same year Neuberg and Langstein (1903) gave alanine to starving rabbits, and not only obtained a subsequent increase in the liver glycogen, but also recovered lactic acid in the urine.

More striking results were obtained by Rosenfeld (1912). He fasted dogs five days, under which conditions he assumed that no more than one per cent of glycogen remains in the liver. Then he fed the animals meat, casein, or glucose. In all cases there was a marked increase in the liver glycogen—as high as 6.2 per cent after meat, and 2.9 per cent after casein. Referring to the fact that in Pflüger's experiments with a fat diet, the liver becomes glycogen-poor, he concluded that glycogen was being formed from protein.

In a very recent paper, Wilson and Lewis (1930) have demonstrated the deposition of glycogen in the livers and whole bodies of white rats, fed with gelatin, casein and glycine, as well as alanine, whose effect upon glycogen deposition was more rapid than that of glycine or glutamic acid.

Pollack (1922) has injected 1 gram each of certain amino acids intravenously in starving rabbits. Glycine, alanine, and aspartic acid resulted in a definite hyperglycemia, as did uneven carbon chain fatty acids. There is, of course, the possibility that the sudden introduction into the blood stream of certain amino acids may cause a temporary flooding of the blood with sugar due to nervous influence upon the liver, but the fact that leucin, as well as even-carbon chain fatty acids had no effect invites the presumption that a new formation of sugar had actually taken place.

In this connection the observations of Parnas and Wagner (1922) are of interest. In a young girl suffering from a liver tumor the blood sugar under fasting conditions was so small in amount as to be scarcely measurable. Following ingestion of carbohydrate or protein, the blood sugar reached normal levels, but not after the ingestion of fat. Again, as in the case of Pollack, this can only be regarded as indicative.

But even if one leaves out of consideration the data on blood sugar, the evidence, practically uncontradicted, leaves little doubt that the ingestion of protein can affect the deposit of glycogen in the normal animal.

b. Calorimetry and the C and N balance. The implication of this, namely, that a conversion of protein to carbohydrate takes place, is strongly supported by the experiments in Lusk's laboratory. The work of Williams, Riche and Lusk (1912) has already been cited in another section (protein to fat). These investigators gave 1200 grams of meat to a dog, and observed the gaseous exchange, the heat production, and the carbon and nitrogen balance over a period of 24 hours thereafter. When 700 grams of meat had been given to the same dog, there had been

very slight evidence of carbon retention, but upon administering the larger quantity, the retention of non-nitrogenous carbon became marked. The authors concluded that this deposit was in the form of glycogen for these reasons: 1, that the observed O_2 consumption agreed with the theoretical calculated on the assumption that the carbon deposit was sugar; 2, that the direct heat production agreed with the theoretical on this basis; 3, that the respiratory quotient tended to fall below the theoretical quotient for protein, which is explicable if an oxygen-poor substance is being converted to an oxygen-rich one. (The latter was not constantly observed during individual periods, but the R.Q. of the entire period was 0.79.) The following table shows the results of a three hour period during the height of carbon retention.

The table is practically self-explanatory, particularly if one reverts back to the discussion of similar experiments in the section dealing with

TABLE 4

(Williams, Riche and Lusk)

The effect of administering 1,200 grams of meat to a dog

	FOUND	CALCULATED (C RETAINED = 3.14 GRAMS)	
		If retained as glucose	If retained as fat
Calories (direct)	122.11	122.86	113.32
Oxygen (grams)	40.35	40.01	36.63
R.Q.	0.77	0.77	0.85

the conversion of protein to fat. There can be no doubt whatever that a retention of carbon actually occurred. Over the long period studied, moreover, the error in assuming the nitrogen determination to represent the actual amount of protein deaminized is reduced to a minimum, so that one can assume beyond question that this carbon, consisting of 13.8 grams over a period of fourteen hours, was non-nitrogenous carbon. Unless one makes the improbable assumption, unsupported by a scintilla of evidence, that this carbon was in the form of unknown intermediary products, it must be granted that it was in the form of carbohydrate or fat. The data indicate the former, and support the evidence obtained from experiments on glycogen deposit.

Similar experiments were carried out by Atkinson, Rapport and Lusk (1922). This investigation was primarily concerned with the formation of fat from protein, but it was incidentally observed that with increasing

quantities of meat fed, there was a change in the character of the deposited carbon. When less than 1000 grams were given, the R.Q. of the deposit indicated that it consisted of a mixture of carbohydrate and fat, as follows:

Meat, 800 grams	R.Q. = 0.83
Meat, 900 grams	R.Q. = 0.96
Meat, 800 grams	R.Q. = 0.84
Meat, 800 grams	R.Q. = 0.86
Meat, 800 grams	R.Q. = 0.86
Meat, 800 grams	R.Q. = 0.83

Only when more than 1000 grams were given was a material laid down in the body whose R.Q., if oxidized, corresponded to that of pure fat. A similar observation was made by Weiss and Rapport (1924).

Taking these calorimeter experiments, with their evidence upon carbon retention in the form of carbohydrate, together with the data already cited showing the effect of protein feeding upon glycogen deposition, it is clear that *in the normal animal* protein can be converted to sugar. It is well to stress this point, because too often the assumption is made that the proof of this conversion rests entirely upon the evidence obtained in diabetes.

One question, perhaps, can be raised at this stage. The experiments so far quoted deal with the ingestion of a diet which is top-heavy with proteins or amino acids. Can we say, as a result of them, that the conversion of protein to sugar or its intermediary metabolic products is normally and constantly going on in the animal body? It must be admitted that these experiments do not furnish such proof. Incidentally, however, the objection mentioned probably holds for every form of conversion of the major foodstuffs, even for such a firmly established conception as that of the formation of fat from carbohydrates. For the further evidence that we have we must turn to the indirect and perhaps equally equivocal information furnished chiefly by the experiments in diabetes. Such evidence as exists for the change which we are now considering should be regarded as in the nature of *legal* proof—of proof beyond *reasonable* doubt on the data at hand. We turn, then, to the confirmatory results in diabetes.

II. Evidence from the Diabetic Animal. a. General confirmation of the conversion of protein to carbohydrate. The evidence obtained from diabetic animals for the most part assumes the conversion and is concerned chiefly with its extent. However, we may first cite general

evidence that the conversion takes place. Külz (1876) gave casein to a young severe diabetic who had been "for a long time" on a diet of meat, eggs and clear coffee. During the experimental periods the man received bouillon, meat extracts and salts to which the casein was added. The results follow:

PERIOD I		PERIOD II	
Casein in 24 hours	Urinary sugar in 24 hours	Casein	Urinary sugar
grams	*grams*	*grams*	*grams*
200	79.0	200	66.0
240	70.1	240	65.7
300	87.1	300	96.7
500	137.4	500	126.9
		240	86.6

On receiving 1240 grams casein in each of the two series, the excreted sugar was, respectively, 373 grams and 355.3 grams, a difference of only about 5 per cent. Külz drew no conclusions except that giving casein was followed by increased sugar excretion, but the data are striking, and the inference is not invalidated by Pflüger's criticism that a 50 kilo man might contain 2000 grams of glycogen from which the sugar might have come.

In his earliest papers on phlorhizin diabetes, Mering (1886) was of the opinion that protein feeding had no effect on the sugar excretion (but he also thought the ingestion of sugar to be equally without effect). In his later work (1887, 1888, 1889) he changed his views, and on obtaining a D/N ratio of 3.3/1 in the urine, came to the conclusion that $\frac{2}{3}$ of the protein molecule was convertible to glucose. Moritz and Prausnitz (1890) found that the ingestion of sugar and protein increased the sugar excretion in phlorhizinized dogs, while that of fat did not. Reilly, Nolan and Lusk (1898), concerning whose experiments we shall have more to say in relation to the amount of sugar obtainable from protein, made a similar observation. Lüthje (1900) observed in a human diabetic that the sugar excretion increased enormously on a high protein diet, and sank to little or nothing on a fat diet (the two extremes were 0 and 112.5 grams urinary sugar). In 1905 he gave a depancreatized dog casein for one month and observed the excretion of 1176 grams of sugar, of which a maximum of only 257 grams could have existed preformed in the body. Moreover, the sugar excretion increased with the amount of protein ingested. Pflüger (1904) had objected to this con-

clusion in one of his numerous polemics, but finally admitted that sugar could derive from protein because of his own experiments with Junkersdorf (1910). In these experiments on phlorhizinized dogs, the glycogen content of the liver rose from an average of 0.56 per cent in starvation to an average of 6.46 per cent after codfish feeding—11.5- to 18-fold increase. The muscle glycogen also increased 5-fold. This was out of all proportion to the glycogen which could have been present in the diet. Pflüger also found that the liver glycogen content increased enormously after administering glycine.

He had always maintained (1892, 1903, 1905, 1, 2) that if extra sugar appeared as a result of the ingestion of protein, it was simply because protein by its own combustion spared the combustion of both carbohydrate and fat. As he assumed diabetes to be due to an excessive production of sugar with no impairment of the oxidative mechanism, the sparing of sugar combustion by protein feeding would result in an increased quantity of the former left unburned—hence appearing in the urine or deposited as glycogen. But in the above experiments, easily oxidizable alcohol resulted in no increased sugar excretion, nor did fat when ingested in large quantity, although both of these substances could also spare the combustion of sugar. By these facts Pflüger was finally led to discard his opposition of many years.

Of the more recent investigations, one may quote those of Csonka (1916), in which the blood sugar of a phlorhizinized dog was increased by 200 grams of meat "isoglucogenic" with 20 grams of glucose though to a less extent than by that amount of glucose itself. Lard—100 and 143 grams—had no effect. A similar observation was made by Labbé and Nepveux (1922) on human diabetics. Very recently, Hanisch and Junkersdorf (1927) noted that the hypoglycemia in phlorhizin diabetes could be obviated by a high protein diet.

The general evidence above quoted indicates that in diabetes, whether human or due to the depancreatization or phlorhizinization of animals, there is evidence of a formation of sugar as a result of the ingestion of protein. This evidence confirms the results obtained from the normal animal. It would be going too far, perhaps, to say that there is absolute proof of the conversion under the usual conditions of mixed feeding, but the presumption that it is a normal occurrence under these circumstances is greater than for any other of the conversions with which we are concerned in the present paper. The next question is: what amount of sugar can be obtained from protein? Most of the work in this field has been directed towards the answer to that question, and to the probable mechanism of the process.

b. The amount of sugar obtainable from protein. 1. *The D/N ratio.* The early work of Mering (1887) in which he found a D/N ratio of 3.3/1 in the fasting phlorhizinized dog, has already been quoted. This usually followed single injections of phlorhizin, and therefore too much weight can not be attached to the observation. Shortly after, Minkowski published D/N ratios obtained in fasting depancreatized dogs. Since then, many such observations have accumulated. For the sake

TABLE 5

*The D/N of starving diabetic animals**

OBSERVER	SUBJECT	TYPE OF DIABETES	D/N
v. Mering (1887)	Dog	Phlorhizin	3.3
Reilly, Nolan and Lusk (1898)	Dog	Phlorhizin	3.67 (3.38–3.90)
Halsey (1899)	Dog	Phlorhizin	3.1–4.2
Loewi (1902)	Dog	Phlorhizin	3.7–4.2
Janney and Csonka (1915)	Dog	Phlorhizin	3.43
Stiles and Lusk (1903)	Dog	Phlorhizin	3.40–3.89
Arteaga (1901)	Cat		2.98
Lusk (1901)	Goat		2.88
Benedict and Lewis (1914)	Man		3.68
Minkowski (1893)	Dog	Pancreatic	2.66–3.16
Pflüger (1905)	Dog	Pancreatic	0–2.8
Campbell and Markowitz (1926)	Dog	Pancreatic	2.05–3.75†
Macleod and Markowitz (1926)	Dog	Pancreatic	1.7–5.9†
Chaikoff, Macleod, Markowitz and Simpson (1925)	Dog	Pancreatic	0.91–3.41
Chaikoff (1927)	Dog	Pancreatic	2.8–6.2
Falkenhausen (1925)	Dog	Pancreatic	2.89

* I have excluded the very high ratios of Hartogh and Schumm (1901), because, as Lusk has shown (1928) they are hardly to be taken seriously.

† Excluding two observations on the 3rd day of starvation.

of brevity, the most important of these observations on the starving animal may be set down in a table.

In addition to the data in the table, there are various observations upon fasting severe diabetics (a list of these is given in Dubois' *Basal Metabolism in Health and Disease*, 1927). Here the tendency for the highest D/N ratios to approach that of the phlorhizinized dogs is striking. Another table will show the D/N ratios that have been obtained

by various investigators upon feeding protein, or mixtures of protein and fat, to diabetic animals.

These two tables (table 5 and table 6) present certain points of considerable interpretative difficulty. It is clear that taking individual observations, there is a certain amount of divergence even in the case of the same animal suffering from the same type of diabetes. Thus, in the experiments of Ringer and Lusk (1910) many of the ratios in the starving phlorhizinized animal correspond very nearly to the Minkowski

TABLE 6

The D/N of diabetic animals on a "carbohydrate free" diet

	SUBJECT	DIET	TYPE OF DIABETES	D/N
Reilly, Nolan and Lusk (1898)	Dog	Gelatin	Phlorhizin	3.54–3.69
Loewi (1902)	Dog	Meat and fat	Phlorhizin	3.1–5.9
Stiles and Lusk (1903)	Dog	Pancreatic digest of meat	Phlorhizin	2.4
Knopf (1903)	Dog	Meat	Phlorhizin	2.76
Knopf (1903)	Dog	Meat and fat	Phlorhizin	2.8–3.1
Rohmer (1910)	Dog	Meat	Phlorhizin	3.2–3.7
Rohmer (1910)	Dog	Casein	Phlorhizin	3.5–3.8
Janney (1915)	Dog	Various proteins	Phlorhizin	3.1–4.5
Janney and Csonka (1915)	Dog	Meat	Phlorhizin	3.3–3.6
Lüthje (1904)	Dog	Meat	Depancreatized	2.1–3.3
Mandel and Lusk (1904)	Man	Meat and fat		3.66
Lüthje (1905)	Man	Meat and fat		3.63
Greenwald (1913)	Man	Meat and fat		3.66
Wilder, Boothby and Beeler (1922)	Man	Meat and fat		3.98

ratio of 2.8/1. Some of this may doubtless be explained on the basis of differences in the rate of glucose and nitrogen excretion, and it is true, as a rule, that even when the same observer reports varying individual D/N ratios, the average of his observations is comparable with the average of other observers. For example, the average of Loewi's D/N of 3.1 to 3.9 on giving meat and fat to a phlorhizinized dog corresponds fairly closely to Stiles and Lusk's average ratio of 3.65, and while Lüthje's range of D/N ratios on giving meat to a depancreatized dog was from 2.1 to 3.3, the vast majority of his observations lay between

2.7 and 3.0. In view of the above mentioned difficulty of partitioning the carbon and nitrogen metabolism of 24 hour periods, it is perhaps fairer to accept more inclusive averages as representing the true D/N ratio.

When this is done certain facts stand out, namely, the usual close correspondence between the D/N ratios 1, of starving phlorhizinized dogs and man; 2, of human diabetics on a "carbohydrate-free" diet. (The ratios given in the latter case are of course the highest observed except for those isolated ones which have justly been called into question on account of the ever-present possibility of surreptitious ingestion of carbohydrate.) In each of these instances the observed D/N is in the neighborhood of Lusk's 3.65/1 ratio. Such a ratio implies, if it be taken to represent the amount of sugar derivable from protein, a yield of 58 grams of glucose for every 100 grams of protein. In the majority of cases a diet of meat or of meat and fat given to a phlorhizinized dog also results in this ratio.

On the other hand, in the starving depancreatized dog the ratio of Minkowski, namely, 2.8/1, has been generally accepted until recently, and has been supported to some extent by the observations of Lüthje on depancreatized dogs fed with meat. The starving phlorhizinized cat and goat also give this ratio, which ratio would imply a yield of 45 per cent of glucose from the protein molecule.

Geelmuyden (1923), and more recently Macleod (1928), have seriously attacked the use of the D/N ratio as indicating the origin of sugar from protein alone. In many cases Macleod and his co-workers have observed low ratios in depancreatized animals. In still others, they have noted ratios as high as 6/1 and more. Still there lurks in the averages of the results the possibility of reconciling them with the Minkowski ratio. Whether or not one accepts the criticism of these investigators will depend upon one's analysis of their data, which too often, I fear, may be coloured by one's prejudices. I will leave the further discussion of this subject until the section on the conversion of fat to carbohydrate, only stating at present my belief that the ratios approximating 2.8/1 and 3.65/1 may be accepted as a proper basis for arriving at the amount of sugar obtainable from meat protein.

But if so, which ratio is the more nearly correct one, recognizing each as only an approximation? The difference between the two remains difficult of explanation. Nothmann (1925) and Rose (1916) have advanced interesting theories in this connection, which imply a greater oxidation of sugar in depancreatized dogs than in the phlorhizinized

animals. On the other hand, evidence has accumulated to indicate that in phlorhizin diabetes the failure to oxidize sugar is not complete. The solution of this mystery is not at hand. The tendency in America, at least, is to assume that of 100 grams of meat protein, 58 grams may be converted to sugar, corresponding to the D/N ratios of phlorhizinized dogs and of apparently completely diabetic men. Upon this basis, for example, is calculated the ketogenic-antiketogenic ratio advanced by Shaffer (1921, 1922) and developed by him and others later. It is not at all certain that this is correct, though for practical clinical purposes the possible error is of little significance.

Various proteins yield different amounts of sugar. The following table, taken from Janney (1915), illustrates this difference. At one time such a distinction would have been attributed to the varying sugar complexes bound to the protein molecule. It is now recognized as a

TABLE 7

(Janney)

Glucose derived from protein (as estimated by the extra sugar excretion upon administering these proteins)

PROTEIN	CASEIN	OVALBUMEN	SERUM ALBUMEN	GELATIN	FIBRIN	EDESTIN	GLIADIN	ZEIN
Glucose yield in per cent.	48	54	55	65	53	65	80	53
D/N Ratio.....	3.08	3.49	3.48	3.59	3.16	3.48	4.53	3.29

function of the different amino acid composition of the proteins, and the fact that the individual amino acids yield sugar in different amounts.

2. *The sugar from individual amino acids.* Friedrich Müller and Seeman (1899) first pointed out (though not on entirely correct premises as to the sugar forming capacity of individual amino acids) that one must look for the origin of sugar from protein not in the preformed sugar in the protein molecule, but in the split-products of amino acid metabolism after deaminization.

Halsey (1899) showed that leucine did not increase the sugar excretion in phlorhizinized dogs. In 1904 he reported further experiments with this amino acid, which, while not entirely conclusive, tended to confirm his earlier result.

Nebelthau (1902) found that asparagin caused a great increase in sugar excretion in dogs that were depancreatized or had a minimum of pancreas left.

Neuberg and Langstein (1903) gave 20 to 30 grams of alanine to starving rabbits, with a consequent increase in liver glycogen. They also recovered lactic acid in the urine, and postulated that the lactic acid was an intermediary step in the conversion.

Knopf (1903), in the course of experiments with phlorhizinized dogs, repeated Nebelthau's experiments with asparagin, finding an increase in sugar excretion. At the same time he obtained negative results on giving urea, showing that the effect was due to the non-nitrogenous portion of the molecule.

Kraus (1904), after poisoning cats with phloretin, gave the animals alanine, and from the extra sugar excretion and the glycogen found in their bodies came to the conclusion that alanine was a source of sugar.

Embden and Salomon (1904) performed experiments on two depancreatized dogs. These were fed with the leanest possible meat, to which on certain days alanine was added. In one dog the sugar excretion, which had amounted to 16 to 19 grams daily on the meat diet, jumped to 29 grams on adding 14 grams of alanine. In the other dog an excretion of 2 to 5 grams of daily sugar was increased to 19 grams when 20 grams of alanine were added to the food. In this experiment, more than half the weight of the alanine appeared as extra sugar. These investigators also (1905) gave sodium lactate, glycine, and asparagin to depancreatized dogs, and obtained large increases in the sugar excretion.

Glaessner and Pick (1907) observed a large excretion of sugar in phlorhizinized rabbits upon the ingestion of alanine, glycine and asparagin. They also obtained some evidence of extra sugar excretion with glutamic acid and leucine, though the effect was slight. Incidentally, they noted that when the animals had previously been starved, alanine had a small effect upon the sugar excretion and glutamic acid none at all, and raised the question whether under these circumstances there might be a retention of the nitrogen-free portion. This observation has never been substantiated.

Up to this time, no serious attempt had been made to quantitate the sugar obtainable from amino acids. In 1908, Lusk investigated the sugar forming power of glutamic acid in the phlorhizinized dog, and concluded that three of the five carbon atoms could enter into the formation of glucose.

Similar experiments were performed by Ringer and Lusk (1910) with a variety of amino acids. They found that the non-nitrogenous portions of glycine and alanine were completely convertible to sugar; and that of the four carbon atoms of aspartic acid, three could be utilized

for the formation of carbohydrate. They also confirmed Lusk's observation in regard to glutamic acid, and obtained negative results with tyrosine.

Dakin (1912) found an extra sugar excretion on giving proline comparable to that obtained by Lusk with glutamic acid, and concluded that three of the five carbon atoms went towards the formation of glucose. In a paper immediately following (1913), he extended these observations on phlorhizinized dogs to other amino acids. In some cases he

TABLE 8
(Dakin)
Glucose from amino acids

SUBSTANCE	INCREASED GLUCOSE EXCRETION WHEN GIVEN TO A GLYCOSURIC DOG
Glycine	+
Alanine	+
Serine	+
Cysteine	+
Aspartic acid	+
Glutamic acid	+
Hydroxyglutamic acid	+
Ornithine	+
Arginine	+
Proline	+
Valine	−
Leucine	−
Isoleucine	−
Lysine	−
Histidine	−
Phenylalanine	−
Tyrosine	−
Tryptophane	−

obtained slight increases in the sugar excretion, but called these doubtful cases negative. He formulated his own results and those of other investigators in the following table, to which I have added β-hydroxyglutamic acid, which Dakin (1919) discovered and which he found to form sugar from three of its carbon atoms.

Dakin drew the general conclusions, among others, 1, that all 2, 3, 4, and 5 carbon amino acids furnish glucose freely, except valine; 2, arginine is the only acid with more than 5 carbons that furnishes glucose freely (this coming from the ornithine moiety); 3, all straight chain

acids except lysine yield sugar, while those with branched chains do not; 4, proline is the only cyclic acid yielding much glucose. (A résumé of the fate of the amino acids may be found in Lusk (1928, p. 222, ff.).)

On the basis of the above quoted evidence, accumulated, as it happens, almost entirely from experiments on phlorhizinized animals, and the estimated amounts of each amino acid in the molecule, the following calculation can be made as to the probable yield of sugar in different

TABLE 9

Sugar obtainable from different proteins

GLUCOGENETIC AMINO ACIDS	MUSCLE PROTEIN*		GELATIN†		CASEIN‡	
	Per cent in molecule	Carbon available for glucose	Per cent in molecule	Carbon available for glucose	Per cent in molecule	Carbon available for glucose
Glycine	4	18	25.5	70	0.45	2
Alanine	8.1	42	8.7	30	1.85	9
Aspartic acid	10.6	30	3.5	9	4.1	12
Glutamic acid	22.3	72	5.8	12	21.77	57
Hydroxyglutamic acid					10.5	24
Proline	8.0	24	9.5	27	7.63	27
Hydroxyproline			14.1	33	0.23	3
Arginine	11.5	33	8.2	15	3.81	9
Cystine						
Serine						
Total		237		199		146
Glucose equivalent in mols		39		33		24
Molecular weight of glucose		7,020		5,940		4,320
Minimum molecular weight of protein	16,005*		10,300†		12,800	
Glucose obtainable from protein	44 per cent		58 per cent		36 per cent	

* According to the formula of Loewy (1911).

† According to Cohn, Hendry and Prentiss (1925).

‡ According to Cohn, Hendry and Prentiss.

proteins. As will be seen, it differs appreciably from the one given by Janney.

In the case of meat protein, this table, like the one given by Lusk (1928, p. 250), indicates the formation of 44 per cent sugar from the molecule, which corresponds to the Minkowski ratio. The Lusk ratio of 3.65/1 implies an extra source of sugar in meat protein, the origin of which is obscure.

It suffices to say, however, that unless one agrees with Macleod that the D/N ratios found in starving or fat-fed diabetic animals do not represent accurately the amount of sugar obtainable from body protein, there can be hardly any question that, within the limits (at present, unfortunately, rather wide) set forth above, a definite and ascertainable quantity of sugar can be formed from protein, that is to say, from certain of the amino acids in the protein molecule.

3. *The diabetic respiratory quotient.* The interpretation of the respiratory quotient in complete diabetes introduces certain difficulties. In the present connection the lowest quotients which have been obtained under trustworthy conditions are worthy of examination to see whether they are compatible with the conception of the formation of sugar from protein. In the phlorhizinized animal fasting respiratory quotients in the neighborhood of 0.69–0.71 are the rule: Wierzuchowski (1926); Deuel, Wilson and Milhorat (1927); Rapport and Ralli (1928). These R.Q.s, lower than the usual fasting quotients of normal animals, are not inconsistent with the oxidation of fat, and of protein with the failure of combustion of most of the carbohydrate moiety of the latter.

Dubois (1927) has published a table of quotients obtained by many observers in human diabetic patients, either fasting, or on a protein-fat diet. Practically all of these R.Q.s are above 0.64 (occasional quotients as low as 0.6 have been found under apparently valid conditions). The question arises as to whether such quotients are compatible with the origin of sugar from protein but not from fat. Magnus-Levy (1905) who himself noted a single quotient of 0.613 in a diabetic man, calculated that on the assumption that 60 per cent of body-protein was convertible to sugar, such a quotient could be obtained from the combustion of protein in a diabetic. This, of course, takes no account of the fat metabolism. None of Magnus-Levy's average quotients was below 0.64 and the majority obtained by him, as well as by other observers, were well above this figure (0.67 to 0.71). As Magnus-Levy pointed out, the problem is complicated by the factor of ketosis, and he calculated that if protein yielded 60 per cent sugar, and an individual utilized 100 grams of protein and 250 grams of fat, producing at the same time 20 grams of β-hydroxybutyric acid, an R.Q. of 0.682 might result. Geyelin and DuBois (1916) observed a respiratory quotient of 0.69 in a diabetic when his D/N ratio was 3.9/1; and Wilder, Boothby and Beeler (1922) obtained the same quotient in a woman whose D/N at the time was in the neighborhood of 3.65 (as high as 3.98). These observers noted the interesting fact that the R.Q. consistently dropped after the ingestion of protein—as low as 0.64 to 0.66 after three hours.

None of these quotients is inconsistent with the origin of sugar from body protein to the extent of 58 to 60 per cent of the molecule. On the assumption that only 44 per cent of body protein is available for glucose production, quotients as low as 0.64 would be more difficult of explanation without invoking the formation of sugar from fatty acids, were it not for the factor of acidosis and the known difficulty of being certain of individual R.Q.s, even under the best conditions, to within 0.02.

We shall return to this subject in the section on the conversion of fat to carbohydrate. It suffices here to state that the diabetic R.Q. does not invalidate, and at present tends to confirm, the production of sugar from body-protein to the extent of 45 to 60 per cent of the molecule, with a leaning toward the latter.

III. The Theory of the Conversion. The theory of the conversion of protein to carbohydrate—or rather the conversion of amino acids to carbohydrate—is discussed in detail in the papers by Lusk (1910), Dakin and Dudley (1914) and in Lusk's book, *The Science of Nutrition.* We are here concerned chiefly with the evidence that shows the formation in the body of intermediary products common to both amino acid and carbohydrate metabolism, and with the possibility of the synthesis of glucose from such compounds. The nature of the conversion depends primarily upon the type of deaminization of the amino acids. Originally it was supposed that the deaminization was hydrolytic, with the type reaction:

$$R\cdot CHNH_2\cdot COOH + H_2O \rightarrow R\cdot CHOH\cdot COOH + NH_3$$

Thus Neuberg and Langstein (1903), on giving alanine to starving rabbits, recovered lactic acid in the urine.

Neubauer (1909), on the basis of experiments with the aromatic acids and particularly tyrosin and phenylalanine, formulated the theory that the amino acids were converted to their corresponding keto acids by oxidative deaminization:

$$R\cdot CHNH_2\cdot COOH + O \rightarrow R\cdot CO\cdot COOH$$

This conception was supported by the experiments of Knoop (1910) on the conversion of amino to ketonic acids in the organism, and the reversal of this process. (The reaction might now be written in accordance with Wieland's dehydrogenation.)

Embden and Kraus (1912) observed the formation of lactic acid in the perfused liver on adding alanine (or glucose) to the perfusate. This did not lead them to deny the oxidative deaminization of Neubauer,

however, which they admitted as occurring "in all probability." Indeed, neither of these conceptions entirely excludes the other, although in the case of the aromatic acids Neubauer was able to show that the corresponding hydroxy acid did not follow the pathway of metabolism of the amino acid, while the keto acid did.

Dakin and Dudley (1913, 1, 2) emphasized still another derivative of deaminization, namely, the group of ketonic aldehydes, stating: "it appears probable that α-ketonic aldehydes may represent the first step in the metabolism of the amino acids." This would be in harmony with Neubauer's conception, for the keto-aldehyde would need only to be oxidized to the acid. They also showed the interconvertibility of certain -amino acids, -hydroxy acids and -ketonic aldehydes, thus,

$$R\cdot CHOH\cdot COOH \rightleftharpoons R\,CO\cdot CHO + H_2O$$
$$R\cdot CHNH_2\cdot COOH \rightleftharpoons R\,CO\cdot CHO + NH_3$$

methyl glyoxal was produced from both alanine and lactic acid; and glyoxal from glycine and glycollic acid. On the basis of the production of the ketonic aldehyde, and subsequent CO_2 cleavage, the formation of sugar from 3 of the 4 carbon atoms of aspartic acid, as shown by Ringer and Lusk, could be easily explained:

$$\begin{array}{lll} COOH & COOH & \overline{CO_2} \\ CH_2 \rightarrow & CH_2 \rightarrow & \overline{CH_2} \\ CHNH_2 & CO & CO \\ COOH & CHO & CHO \end{array}$$

The point to be considered here is that in the case of such an amino acid as alanine, for example, which has been most extensively studied, any or all of these pathways might yield sugar, for lactic acid, methyl glyoxal and pyruvic acid are all convertible into each other, and into sugar (although not, apparently, with equal facility).

Although in many if not all of the amino acids, the exact mechanism of the splitting off of their non-nitrogenous moieties is still obscure, it is clear that in the case of the sugar forming ones the possibilities exist for the synthesis of dextrose and glycogen from these intermediary products. It does not follow from this alone, of course, that sugar is actually being synthesized normally and constantly in the ordinary catabolism of protein. For the proof of this we must revert back to the earlier portions of this section.

Recently Mann and Magath (1922, 1923), in their studies on the

effects of liver extirpation, have observed that the resulting hypoglycemia, while relieved by administration of glucose, is not affected favorably by intravenous injection of glycine. This would appear to prove that the conversion of the amino acid to sugar is dependent upon the integrity of the liver. On the other hand, it does not seem to me to prove that the conversion is entirely a function of the liver. In the first place, Mann and his co-workers have themselves shown that deaminization does not occur upon liver extirpation, and the lack of sugar formation may be due to the failure of deaminization. In the second place, an animal with as disturbed a metabolism as the dehepatized one may have lost the power to synthesize sugar, apart from any specific liver function. Certain evidence seems to indicate that a depancreatized animal, shortly before death, may also lose this ability.

IV. The Thermodynamics of the Process. It is difficult to discuss this phase of the subject on any but highly theoretical grounds, because of the incompleteness of the data. Thus, the heat of combustion of many of the amino acids has not been determined. In the conversion of most of the glucogenetic amino acids the process is endothermic; in the case of cystine it appears to be exothermic. The equation in the case of alanine, following Lusk (1915), may be written:

$$2\ CH_3 \cdot CHNH_2 \cdot COOH + H_2O + CO_2 = C_6H_{12}O_6 + CON_2H_4.$$

Thermodynamically, it may be represented as follows:

$$2\ CH_3 \cdot CHNH_2 \cdot COOH + O_2 = 2\ CH_2 \cdot CO \cdot COOH + 2\ NH_3.$$
$$776\ \text{Cals.}\ (-44\ \text{Cals.}) \qquad 549.0\ \text{Cals.}\ +183\ \text{Cals.}$$
$$2\ CH_3 \cdot CO \cdot COOH + 2\ H_2 = C_6H_{12}O_6$$
$$549\ \text{Cals.}\ (+132\ \text{Cals.}) \qquad 681\ \text{Cals.}$$
$$2\ NH_3 + CO_2 = NH_2 \cdot CO \cdot NH_2$$
$$183\ \text{Cals.}\ (-31\ \text{Cals.}) = 152\ \text{Cals.}$$

The whole reaction is therefore approximately 132 Cals. −75 cals., or 57 cals., endothermic. Taking this as a closed thermodynamic system, therefore, it will be seen that the energy exchange in the conversion is relatively small. When glycogen was actually being deposited as a result of protein ingestion, in the experiments of Lusk and his co-workers, the heat production bore no relation, in fact, to the extent of this deposit. It must not be forgotten, however, that when sugar is not being oxidized there is a loss of energy available from the protein molecule to the extent to which the sugar that is formed from protein fails of combustion. This may be considerable, and cannot be neglected in calculating the heat value of protein to the diabetic.

From another point of view the conversion of amino acid to sugar has been invoked to explain the specific dynamic action of protein. Aubel (1924, 1925, 1927), using alanine as an example, postulated the presence of a coupled exothermic reaction, namely, the oxidation of glucose, to an extent which not only provides the energy for the conversion of alanine to glucose, but also yields an excess which, being dissipated as heat, represents the specific dynamic action. Geelmuyden (1925) goes even farther, and assumes that carbon appertaining to the non-nitrogenous moiety of all of the amino acids is convertible to glucose. When, in the case of a particular amino acid, this reaction is exothermic, this in itself may be a source of the specific dynamic action; otherwise, the excess energy is derived from the oxidation of glucose.

Apart from the fact that Geelmuyden discards the evidence showing that many amino acids are not glucogenetic, the theory in common with that of Aubel, implies an obligatory oxidation of glucose. These theories bring into bold relief the pitfalls in postulating chemical reactions on the basis of thermodynamic data. For example, as Lusk has shown, glycine and alanine exert the same specific dynamic action in the diabetic as in the normal animal, a phenomenon which he and Chambers (1930) have very recently proved to apply to the whole protein molecule.

On the other hand, the general conception that exothermic reactions, coupled with endothermic reactions, and furnishing energy in excess, as Adams (1926) and Chambers and Lusk (loc. cit.) emphasize, might account for the waste heat of protein metabolism, may be sound. The danger lies in assuming specific reactions.

V. Summary. In this section, the evidence that has been considered in relation to the conversion of protein to carbohydrate is overwhelmingly in favor of the view that such a conversion may occur. No physiologist at the present time, indeed, denies this. A large part of the data, it is true, are drawn from experiments upon artificially induced or naturally occurring diabetes, and were this the only proof of the conversion one would have to say as Dakin did in this connection, that "it is of course open to question how closely the normal path of catabolism resembles that observed under abnormal conditions." But in addition to the observations on the diabetic animal there is sufficient amount of evidence to indicate that the process may also go on in the normal animal. That certain of the amino acids are convertible to intermediary products which in turn are convertible to glucose is unquestioned. There are only two controversial points:

1. To what extent is sugar formed from protein? It is clear that different amounts of sugar are derivable from different proteins. Since the individual amino acids vary in their sugar-forming capacity, and since proteins differ in their content of the individual amino acids, this is quite understandable. In the case of the most important protein group in this respect, namely, the body proteins (chiefly the muscle proteins), the amount of available glucose hinges upon the validity of the D/N ratios, especially the Lusk ratio of 3.65/1 and the Minkowski ratio of 2.8/1. Although the view has been advanced that these ratios are not in any sense to be regarded as indexes of the extent of the conversion of protein to sugar, it seems to me that their importance in this connection cannot, on the basis of our present knowledge, be denied. Nevertheless, in view of the unexplained divergence between the two significant, if approximate, figures, the percentage of any protein that is available for glucose formation cannot at present be stated with exactness. It is possible, as the data would seem to indicate, that the maximum glucose formation in any particular case is a function not of the protein in question, but of the animal which is being studied. In man, this maximum would appear at present to correspond, for body protein, to the Lusk ratio.

2. Is there under conditions of normal feeding in the normal animal a production of glucose or glycogen from the non-nitrogenous split-products of the amino acids? This question, I believe, also cannot be definitely answered. In so far as carbohydrate is essential for the bodily processes, both in growth and in adult life, however, such experiments as those of Osborne and Mendel (1924) indicate that even on a practically pure protein diet it can be supplied throughout the life span of an animal. It is even possible, as Paton and Cathcart postulated, and as has recently been restated by Macleod (1928), that carbohydrate from protein may normally be used as fuel for muscular exercise, without concomitant loss of the nitrogenous portion to the body.

Lusk (1928, p. 271) says: "The production of glucose from protein is not an emergency function as some writers maintain, it is a normal function."

THE CONVERSION OF CARBOHYDRATE TO FAT. That carbohydrate can be converted to fat in the animal body has long appeared obvious to the layman who has observed the fattening effect upon stock of a diet of grains or upon human beings of a regimen consisting largely of sugary or starchy foods. That the conversion can, under certain circumstances, occur, no present day physiologist would deny. But the proof

consists chiefly of experiments in which a high-calorie diet containing excessive amounts of carbohydrate has been administered, and we are even now unable to be certain whether it is only under such conditions that the phenomenon takes place.

Liebig first advanced the idea of the conversion, and it was generally accepted until Carl Voit questioned it. In his article in Hermann's Handbuch der Physiologie (1881), Voit reviewed the evidence that seemed to support Liebig in his contention, and says of the latter: "from then on (the time of Liebig), this appeared such a well established fact, that any doubt concerning it was held to be so much nonsense." (Pflüger's later remarks concerning Voit's influence in establishing a general acceptance of the conversion of protein to fat furnish an amusing counterpart to this.) Voit, in fact, believed the evidence for the production of fat from carbohydrate to be unreliable.

The early experiments, it is true, yielded many errors on analysis. Later, this evidence was strengthened by the experiments in which the carbon and nitrogen balance of the food and excreta, together with the actual determination of the fat deposition, were studied. The chief error of these experiments, and one that makes them fall short of absolute proof, was due to the fact that the ethereal extract was assumed to represent the fat quantitatively. Nevertheless, the results were often so striking that even admitting the error, there can be no doubt that the conversion was demonstrated.

I. Experiments on the Direct Determination of Deposited Fat. In the eighties, several researches of this character were performed, animals being overfed with foods containing chiefly carbohydrates. Soxhlet (1881) found in young pigs fed with rice a deposition of as much as 200 grams of fat per day above that which could have come from the fat in the food. Schultze (1882) and Chaniewsky (1884) observed the same phenomenon in geese fed with rice, barley and starch. Tschirwinsky (1883), using growing pigs, and Munk (1885), studying a young dog, made similar observations.

II. Respiration Experiments Involving a Study of the C and N Balance. More convincing data, however, were derived from respiration experiments. A classical example of this, often quoted, lies in the experiments of Meissl and his collaborators, reported by him in 1886. In these experiments two kilos of rice were given daily to a young pig. The respiratory exchange was obtained, and the food intake and excreta analyzed.

The following is an average of four experiments.

PROTEIN RETAINED	C FROM PROTEIN	C DEPOSITED	C AVAILABLE FOR FAT	FAT DEPOSITED
41.5	22.0	249.1	227.1	296.9

As will be seen, there was a retention of N, which, as protein, could account for 22.0 grams of carbon. Since 249.1 grams of carbon were actually deposited, 227.1 grams were available to form fat. Such a daily deposit could hardly be glycogen. As fat, it would amount to 296.9 grams per day. The N excretion could only account for, at the most, 20 per cent of this fat as arising from protein, and the conclusion was inevitable that the remainder must have come from carbohydrate.

Rubner (1886) showed the same thing in a small dog of 6.2 kilos. After fasting, the dog received 100 grams of cane sugar, 85 grams of starch and 4.7 grams of fat for the two days during which the experiment was performed, with the following result.

	grams	
C intake	176.6	
C output	87.1	
	89.5	
Possible from protein	13.0	
	76.5	
Possible deposit as glycogen	34.7	
	41.8	
Probable maximum retention of starch intake as such	10.7	
	31.1	= 40.7 grams fat

Lehmann and Voit (1901) reported experiments done many years before on geese fed with large amounts of rice. The following is an average of experiments performed on six animals. There was no appreciable nitrogen retention.

	grams
In the animal	327.3
Possible from protein in diet	62.6
From starch (total)	264.7
(For 1 day)	18.3
(For 100 gm. starch)	12.4

If the deposited carbon had been in the form of glycogen, the latter would have been at least five times as much as E. Voit showed to be present in a goose fed in a similar manner. Since the protein metabolized could at most account for only one-fourth of the deposited carbon, the latter must have been largely, at least, derived from carbohydrate.

These experiments constitute as nearly certain proof as can be obtained from this type of investigation.

Recently, Ellis and Isbel (1925) and Ellis and Hankins (1926) have shown that the type of fat deposited can be markedly affected by the ration. Hogs fed on brewer's rice and tankage (containing less than one per cent of fat) and corn, lay down a "hard" body fat that is less unsaturated than that which is deposited on a diet containing large amounts of fat (such as peanuts or soy bean). In the latter case there is a large increase in the percentage of linolic acid in the body fat.

Hoffman and Wertheimer (1927) conceive that the conversion of carbohydrate to fat occurs in the fatty tissues themselves. Their evidence for this consists of the fact that ordinarily animals contain practically no glycogen in these tissues, but on a carbohydrate-rich diet following a period of starvation much glycogen is present. They admit the possibility that this glycogen may be transported to the liver, there changed to trioses and thence to fat, and the fat transported to the fat depots, but argue against it because of Bleibtreu's finding that the blood of an animal overfed with carbohydrate is usually fat-poor. This, it is true, is not a very convincing argument, for the determination of blood fat by antiquated methods cannot furnish much in the way of corroboration: and the blood fat, in addition, is not necessarily an index of the amount of fat transportation going on. Moreover, the assumption that the glycogen found in the fatty tissues is ultimately converted to fat at all is purely hypothetical.

III. Experiments Involving a Study of the R.Q. The theoretical combustion respiratory quotient of none of the foodstuffs being greater than unity, it follows that a higher R.Q. than this, if the errors due to changes in the CO_2 content of the body as a result of acidosis, etc., are ruled out, must be due to the conversion of an oxygen-rich substance to an oxygen-poor one. If one could suppose an oxidation of carbohydrate alone, associated with the conversion of protein to fat, the latter might certainly account for a respiratory quotient greater than one. Since such an assumption is, on the basis of the known facts, unwarranted, we are obliged to fall back upon the conversion of carbohydrate to fat to explain the excessively high quotients; conversely, the presence of such quotients

(again assuming the absence of what in this connection is artifact) is used as proof of the conversion. This has appeared to establish a sort of dead-line at an R.Q. of 1.00, to make it seem as though the conversion cannot take place unless this quotient is exceeded. It will be obvious that there is no theoretical reason why this should be so. In fact certain authors, such as Krogh and Lindhard (1920) suggest that the production of fat from carbohydrate may take place at much lower quotients.

It is equally obvious that from the respiratory quotient alone, however, proof of the conversion is not to be expected when R.Q.s of less than unity are observed, for the possible formation of fat from sugar is masked by the fact that such quotients may be partly or wholly combustion quotients.

The earliest evidence from the respiratory quotient is to be found in the work of Regnault and Reiset (1849) and Reiset (1863). These investigators were not primarily concerned with this ratio, but rather with the ratio of the oxygen contained in the excreted CO_2 to the oxygen consumed. This ratio of weights, however, accurately represents the R.Q., and in a few cases where diets rich in carbohydrate were given to fowls and to a boar, R.Q.s as high as 1.05 were obtained. The authors themselves laid no stress upon these.

In 1892 Hanriot reported that he had obtained R.Q.s as high as 1.25 on giving 50 grams of glucose to a fasting man. No details are given, and such a quotient, under the conditions, seems excessive. Hanriot calculates the "excess" CO_2 due to the conversion as the amount above the basal CO_2. This certainly is not justified. In other experiments quotients of 1.08 and 1.10 were obtained in men, after ingestion of glucose. Bleibtreu (1901) observed very high respiratory quotients in geese upon stuffing the animals with grain, the average of six experiments being 1.22, as compared with the starvation quotients of 0.69 and 0.73. In one experiment an R.Q. of 1.38 was obtained during a period of 32 minutes.

In the marmot, Pembrey (1901) obtained quotients as high as 1.4 in the period before hibernation, when large amounts of carbohydrate were being eaten in preparation for the winter sleep. The hibernating animal presents an interesting and peculiar problem, and the converse of the above condition, namely, the low and questionably authentic quotients observed during the hibernation itself, will be discussed in the next section.

Morgulis and Pratt (1913) noted high R.Q.s averaging 1.06, in dogs

on forcing glucose; and Grafe observed a non-protein quotient as high as 1.28 in the dog and 1.26 in the pig on excessive carbohydrate ingestion.

Lusk (1915) gave a dog 70 grams of glucose, and obtained quotients varying from 1.03 to 1.16. The Bleibtreu conversion formula (of which more will be said in a moment) was used, and the agreement of direct and indirect calorimetry argued for the correctness of the assumption that sugar was being converted to a higher fatty acid with the evolution of CO_2.

Rapport, Weiss and Csonka (1924), studying the respiratory metabolism of a stunted young pig whose protein intake had been restricted, found that when the animal was given a large amount of carbohydrate the R.Q. rose in the next few hours to as high as 1.26. High quotients (1.17 to 1.22) were obtained as long as 21 hours after the ingestion of a heavy carbohydrate meal.

Wierzuchowski and Ling (1925), using a young well nourished hog, performed similar respiration experiments, and observed what are probably the highest authentic quotients representing the conversion ever obtained. In one case the R.Q. reached 1.58. At the same time, the rise in the heat production averaged over 100 per cent above the basal level during the first few hours after carbohydrate ingestion. The heat equivalent of the fat formed during the period of observation was $2\frac{1}{2}$ times the basal energy requirements. The results are given in Table 10.

Baumgardt and Steuber (1920) performed experiments upon one of themselves, studying the respiratory exchange and N excretion after a meal containing 1200 to 1500 cals., nearly all of which was supplied by carbohydrate, and observed R.Q.s as high as 1.10. Assuming that roughly 0.4 gram of fat was produced from 1 gram of starch, they calculated that even if all the carbon of protein were converted to fat, this amount, plus the fat in the diet, could only account for half of the estimated fat production.

Recently Lublin (1926, 1927) has performed experiments on non-diabetic patients, in which the R.Q.s rose to as high as 1.14 upon the ingestion of glucose and levulose. This investigator found that the rise in the quotient above unity was facilitated by insulin, and hindered by epinephrin and related substances. He concluded that there was a corresponding influence upon the conversion of carbohydrate to fat. The quotients seem higher than one would expect after the amounts of glucose and levulose which were given (R.Q.s for example, of 1.14 after 50 grams of levulose, and 1.10 after 50 grams of glucose), and the postulated conversion must be accepted with caution.

Wesson (1927) has reported amazing R.Q.s in rats that were given a meal of dextrin following a period of several weeks during which the animals subsisted on a "restricted" diet (consisting usually of dextrin, salts and a small quantity of oatmeal). The test meal of 3 grams of dextrin per 100 grams of body weight was followed by R.Q.s many of which were as high as 1.5. In one instance a quotient of 3.29 was obtained six hours after the meal! The animals were definitely abnormal as a result of their previous diet, and many died during the course of the experiments. The effects of muscular activity and of acidosis, which may very well have complicated the results, do not appear to have been controlled.

IV. The Extent to Which Fat can be Formed from Carbohydrate. There is no doubt that, as the authentic data among the foregoing indi-

TABLE 10

(Wierzuchowski and Ling)

The conversion of carbohydrate to fat in a hog

DIET	TIME AFTER FOOD-HOURS	R.Q.	FAT FROM CARB. PER HOUR	HEAT PRODUCTION (CALS. PER HOUR)	INCREASE OVER BASAL
200 grams starch	3, 4	1.52*	6.9	41.9	
100 grams glucose	2, 4, 5, 6	1.44	6.2	43.8	
	2	1.34	4.8	42.6	
	2, 3	1.37	5.2	42.0	
			5.8 = 55 Cals.	42.7	102 per cent

* R.Q. 3rd hour = 1.58, 4th hour = 1.46.

cate, carbohydrate may be converted to fat. The question arises: To what extent and under what conditions does this process occur?

The maximum amount of fat produced from carbohydrate, as noted by various observers, may be seen in table 11.

The best fat factories seem to be the goose and the pig. Bleibtreu's animal appears to hold the record, though the observation recorded was only for a half-hour period. The maximum R.Q., it will be recalled, was obtained by Wierzuchowski and Ling.

While the amounts of fat produced under the above conditions have been described as the "maximum," there is no certain proof that they actually do not represent a greater amount than indicated, at least in so far as they are inferred from the rise in the respiratory quotient above unity. We commonly make the assumption that a quotient of

unity represents the combustion of carbohydrate, but it cannot be denied, as I have suggested above, that the conversion of carbohydrate to fat may take place at lower R.Q.s. It is equally true, however, that the latter remains a postulate, which may be considered as a possibility or even a probability because we know that fat can be produced from carbohydrate, and there is no *a priori* reason why it should begin only after carbohydrate has become the sole fuel. A similar argument might apply at the other end of the scale—at low quotients—to the reputed conversion of fat to carbohydrate, were it certain that this conversion can be accepted. Of this we shall have more to say later.

The evidence so far presented, it will be noted, has in all cases dealt

TABLE 11

The maximum conversion of carbohydrate to fat

OBSERVER	ANIMAL	FAT DEPOSITED IN 24 HOURS	
		Amount	Per cent of body fat
		grams	
Munk (1885)	Dog	37	0.1
Rubner (1886)	Dog	20	0.3
Chaniewsky (1884)	Goose	19	0.5
Lehmann and Voit (1901, 1885)	Goose	18	0.4
Bleibtreu (1901)	Goose	104*	2.2
Meissl (1886)	Pig	364	0.2
Grafe (1914)	Pig	53	0.3
Rapport, Weiss and Csonka (1924)	Pig	82*	0.3
Wierzuchowski and Ling (1925)	Pig	125*	0.9
Baumgardt and Steuber (1920)	Man	148*	0.25

* Calculated from the CO_2 in excess of an R.Q. of unity.

with conditions in which the formation of fat from sugar has been induced by excessive carbohydrate ingestion. This raises the question whether the conversion can be considered a normal occurrence under the usual conditions of mixed feeding, a question which is related to the point brought up in the previous paragraph in connection with the respiratory quotient.

Unfortunately, the data do not throw very definite light upon this, and the situation is similar to that which we met in discussing the conversion of protein to fat. The chief difference between the two lies in the fact that the evidence in favor of the production of fat from protein is much less voluminous (possibly owing to the difficulty of demonstrat-

ing the phenomenon), and much less apt at present to be accepted without question, than is the corresponding evidence for the production of fat from carbohydrate.

The reviewer confesses that in beginning to write the ensuing paragraph, he was about to say that the conversion of carbohydrate to fat could certainly be regarded as occurring with greater facility than the conversion of protein to fat. Reflection upon this, however, brought to light a question which is raised in table 12.

Examining the data in this table, one is driven to ask whether indeed there is any greater tendency for the conversion of sugar to fat than for

TABLE 12

A comparison of the conversion of carbohydrate to fat and protein to fat

OBSERVER	ANIMAL	WEIGHT	TEST MEAL		FAT RETAINED IN TWENTY-FOUR HOURS	
			Actually given	Glucose equivalent	Total	Per kilo body weight
		kgm.		*grams*	*grams*	*grams*
Protein to fat:						
Cremer	Cat	3.7	450 grams meat	55	7.3	2.0
Atkinson, Rapport and Lusk	Dog	12	1,200 grams meat	150	28.8	2.4
Carbohydrate to fat:						
Rubner	Dog	6.2		185	20.4	3.3
Lusk	Dog	14		70	17.8	1.3
Baumgardt and Steuber	Human	60		300–350	144	2.4
Wierzuchowski and Ling	Pig	14		300	125	7.9

the similar conversion of protein. Per gram of each substance, undoubtedly there is, but if one assumes that the formation of fat from protein passes through carbohydrate, then the discrepancy between the two is very slight. The glucose moiety of ingested protein appears to be as apt to be converted to fat as carbohydrate ingested as such. No doubt species differ in this respect. The results of Wierzuchowski and Ling on a pig, included in the above table, show a much greater tendency to fat formation than is apparent in the protein experiments, but they also betray a marked discrepancy with the carbohydrate experiments on the dog and the human. Bleibtreu's data on the goose would show

a similar difference. Unfortunately, there is no evidence on the conversion of protein to fat in either the pig or the goose to furnish a fair comparison with these experiments.

It would be possible to labor the figures in table 12. They are at best suggestive only. Any validity which may be inherent in them rests upon the glucose equivalents, and perhaps upon the caloric equivalents. A possible fallacy lies in the above mentioned probability that the production of fat from sugar may occur at respiratory quotients less than unity, which would raise the maximum fat obtainable from carbohydrate under the experimental conditions.

But even if one admitted that carbohydrate is more readily convertible to fat than is protein, one would still not be able to state that under the usual conditions of animal existence the conversion takes place. At most, one can only say that with a great excess of carbohydrate in the diet, the production of fat from sugar undoubtedly occurs in all the species that have been studied; in some with greater facility than in others.

In the experiments of Rapport, Weiss and Csonka (loc. cit.), there is evidence that if the animal is subjected to an increasing need for fuel, the conversion of carbohydrate to fat may be diminished. This is shown in table 13.

It appears from this table that as the muscular work required the animal to utilize for fuel more of the carbohydrate with which the body was being flooded, there was a decreasing tendency for this carbohydrate to be converted into fat for storage.

A similar observation has been made by Rapport and Ralli (1928). In certain of the experiments reported by these investigators, a dog was caused to run on a treadmill after a high-carbohydrate meal. The R.Q. of the period of exercise and recovery was compared with the average R.Q. of the resting period before and after. The significant figures in this connection are as follows:

DOG	EXPERIMENT	NON-PROTEIN	
		Resting R.Q.	Exercise R.Q.
6	6	1.041	1.008
7	10	1.011	0.963
8	5	1.023	0.985

V. Theoretical Considerations. a. The thermodynamics of the conversion. The chief difficulty in discussing the thermodynamics of the formation of fat from carbohydrate is that we are not certain of the

chemical equations. We can assume that one starts with glucose, but it is hard to say whether one ends up with the glyceride of palmitic, stearic, oleic, or other fatty acids, or mixtures of them in unknown proportions. Various formulae have been advanced. There are, for example, three formulae in the literature beginning with glucose and ending with stearin, but in one, 12 mols of glucose form 1 mol of stearin; in another, 14 mols of glucose are required; in the third, 19 mols of glucose yield 2 mols of stearin. The latter, the formula of Zuntz, is very often employed. It is 25 per cent endothermic. Another popular formula appears to derive from Hanriot. In it 13 mols of glucose are converted to the tri-glyceride stearo-oleo-palmitin, as follows:

$$13\ C_6H_{12}O_6 = C_{55}H_{104}O_2 + 23\ CO_2 + 26\ H_2O$$
272.1 gm. glucose (1023 Cals.) = 100 gm. Fat (950 Cals.)

TABLE 13

(Rapport, Weiss and Csonka)

Effect of muscular activity on the conversion of carbohydrate to fat

CALORIES PER HOUR	R.Q.	BEHAVIOR
34	1.22	Quiet
38.2	1.13	Restless
46.0	1.01	Much movement

In other words, the reaction is about 7 per cent exothermic. Both reactions assume that the excess oxygen is used to form CO_2, the one from carbon within the glucose molecule, the other, from carbon derived elsewhere. The result is to increase the numerator of the respiratory quotient without affecting the denominator. Bleibtreu, calculating the conversion on the basis that pork fat was being formed (though he happened to be feeding carbohydrate to a goose) and that all of the carbon of the glucose was converted, arrived at the following:

270.06 gm. glucose = 100 gm. fat
1012 Cals. = 950 Cals.

The reaction is exothermic to the extent of 6 per cent, which is the same as that of Hanriot. Almost the same calculation was made by Magnus-Levy (1894) on the assumption that 27 mols of glucose were converted to 2 mols of tri-stearo-oleo-palmitin. If one of these reactions is assumed it is seen that very little heat is involved in the conversion.

This seems to be borne out by the experiments of Lusk (loc. cit.) in which the height of the heat production bore no relation to the height

of the respiratory quotient. Moreover, there was agreement between direct and indirect calorimetry when the Bleibtreu formula was employed, allowing 1.09 cals. for each liter of CO_2 excreted in excess of a respiratory quotient of unity. Similar results were obtained by Rapport, Weiss and Csonka (loc. cit.). In this investigation it was observed that high respiratory quotients might persist for almost 24 hours after a large carbohydrate meal, although the heat production had returned almost to the starvation level (e.g., expts. 43 and 44). The same phenomenon was seen by Wierzuchowski and Ling (loc. cit.).

There is another aspect of this question, namely, the efficiency with which muscular exercise is performed, which belongs more properly to the section on the conversion of fat to carbohydrate, and will be discussed there. The question of the proper thermodynamic equation to write for the formation of fat from sugar must remain an open one for the present.

b. Possible chemical pathways. The pathway of the conversion of glucose to fat is still obscure. It may be assumed that fatty acids are formed, and that union with glycerol then takes place to form the neutral fat. That molecules of hexose may condense to yield fatty acids is not generally believed. It is supposed that glucose breaks down to form either three carbon or two carbon fragments, and that fatty acid synthesis begins at this point. Alternative theories (though they are not mutually exclusive) are.

1. The aldol condensation. This was originally suggested by Nencki (1878), and elaborated by Hoppe-Seyler, Spiro, Magnus-Levy and Leathes. The simplest form can be written:

CH_3	CH_3			CH_3
CHO	CHOH	HH	→	$CH_2 + H_2O$
CH_3	CH_2			CH_2
CHO	CHO	O		COOH
Acetaldehyde	Aldol			Butyric Acid

Or, if aldol should condense with another molecule of acetaldehyde with reduction by hydrogen from, for example, formic acid formed by the conversion of lactic acid to acetaldehyde, then:

CH_3		CH_3		CH_3
CHOH	H_2	CH_2		CH_2
CH_2	→	CH_2	→	CH_2
CHO		CHOH		CH_2
		CH_2		CH_2
CH_3		CHO		COOH
CHO				

A continuation of this condensation would yield longer chain aldehydes, with ultimate oxidation to produce the fatty acid. The formation of unsaturated aldehydes was suggested by Spiro to Magnus-Levy (1889), thus:

$$\begin{array}{l} CH_3 \\ CHO \\ \overline{CH_3} \\ CHO \end{array} \xrightarrow{-H_2O} \begin{array}{l} CH_2 \\ CH \\ \\ CHO \end{array} \xrightarrow{+H_2O} \begin{array}{l} CH_2 \\ CH_2 \\ \\ COOH \end{array}$$

Leathes and Raper (1925) suggest the formation of long chain unsaturated aldehydes by the aldol condensation, with reduction to form saturated aldehydes and final oxidation to the corresponding fatty acid; for example:

$$4\,CH_3\cdot CHO \rightarrow CH_3\cdot CH\cdot CH\cdot CH\cdot CH\cdot CH\cdot CH\cdot CHO + 3\,H_2$$
$$\rightarrow CH_3(CH_2)_6\cdot CHO + O \rightarrow CH_3(CH_2)_6\,COOH.$$

This eight carbon acid has been built up in this manner by Raper (1907).

2. The pyruvic acid—acetaldehyde condensation. This was suggested by Smedley (1912) and Smedley and Lubrzynska (1913). On the basis of Neuberg's splitting of pyruvic acid into acetaldehyde and CO_2 by the carboxylase of yeast, Smedley postulated a similar occurrence in the body, the aldehyde at once condensing with pyruvic acid to form a higher keto acid from which an aldehyde could be formed by decarboxylation, or a fatty acid by oxidation, thus:

$$\begin{array}{l} CH_3 \\ | \\ CHO \\ \overline{CH_3} \\ | \\ CO \\ | \\ COOH \end{array} \xrightarrow{-H_2O} \begin{array}{l} CH_3 \\ | \\ CH \\ | \\ CH \\ | \\ CO \\ | \\ COOH \end{array} \xrightarrow{-CO_2} \begin{array}{l} CH_3 \\ | \\ CH \\ | \\ CH \\ | \\ CHO \end{array} \xrightarrow{+H_2} \begin{array}{l} CH_3 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ CHO \end{array}$$

This final product, butyl aldehyde, could then condense with another molecule of pyruvic acid to continue the synthesis, thus:

$$\begin{array}{l} CH_3 \\ CH_2 \\ CH_2 \\ CHO \\ \overline{CH_3} \\ CO \\ COOH \end{array} \xrightarrow{-H_2O} \begin{array}{l} CH_3 \\ CH \\ CH \\ CH_2 \\ CH_2 \\ CO \\ COOH \end{array} \xrightarrow{-CO_2} \begin{array}{l} CH_3 \\ CH \\ CH \\ CH_2 \\ CH_2 \\ CHO \end{array} \xrightarrow{+H_2} \begin{array}{l} CH_3 \\ CH_2 \\ CH_2 \\ CH_2 \\ CHO \end{array}$$

To this conception, Neuberg and Arinstein (1921) have added another, namely, the condensation of 2 mols of pyruvic acid to form an aldol which may also be an intermediary step in the formation of fatty acid.

The best that can be said now is that until we know more of the intermediary metabolism of both carbohydrate and fat, but particularly of the former, it is impossible to state definitely just which pathways are chosen in the animal body for the conversion of one into the other. (The subject is discussed in Leathes and Raper's monograph, *The Fats*.)

VI. Summary. From the evidence presented, it is clear that carbohydrate can be converted into fat in the animal body. This indeed is not questioned at the present time. The data which prove the conversion, however, are based upon experiments in which the organism is flooded with the metabolites of sugar resulting from the ingestion of large amounts of carbohydrate. The question can therefore be raised whether under the usual conditions, where the animal is subsisting on a mixed diet whose caloric content is not greatly in excess of his immediate needs, the conversion still takes place. A definite answer to this question cannot be given. The problem is not greatly different in this respect, except perhaps quantitatively, from that which is raised on considering the conversion of protein to fat; and even the quantitative difference between the two phenomena—the difference in the facility with which the conversion takes place—is probably not as great as is commonly assumed.

The exact chemical nature of the conversion is still obscure, and even the end products cannot be stated with certainty. As a result, the thermodynamic data are correspondingly ambiguous, though experimental evidence indicates that the process is associated with very little change in energy content.

THE CONVERSION OF FAT TO CARBOHYDRATE. The possible occurrence of this conversion in the animal body is as controversial as the reverse reaction is not. Few matters in metabolism have more engaged the interest of physiologists, and it cannot be said today that the question is settled. It is impossible to write upon the subject without having, in general, a certain leaning in one direction or the other. There is no harm in this, provided it does not lead to unfairness, particularly in the direction of emphasis. This the reviewer has attempted to avoid.

To go into all of the vast literature having an indirect relevance to the subject is beyond the physical possibilities of this paper. The older literature has been adequately reviewed (see general bibliography), and will be referred to only in so far as it has a pertinent relation to more modern work.

It should be recognized at the outset that in discussing the conversion, two questions have to be considered: 1. *Must* fat be converted to carbohydrate in the animal body? 2. *May* such a conversion take place under certain circumstances? The distinction between these would seem to be sufficiently obvious, but frequently there appears to be a confusion with regard to them. The truism cannot be too often emphasized that even if a phenomenon is shown to take place under unusual conditions, it does not follow that it is a necessary, or even a customary, occurrence. This is true even if the process occurs in the animals with which we are at present concerned, namely, the mammals; it is all the more true when comparison with what happens, for example, in plants or micro-organisms, is made, in order to establish the truth or falsity of the conception as applied to the mammalian organism. As has been pointed out in previous sections, the argument by analogy is particularly dangerous in this field. Even if it be admitted that fat may be changed to carbohydrate in plants; in proving the same process in animals, this might as well, in our present knowledge, be neglected. The same applies to the argument based upon the general tendency to reversibility of chemical reactions in the body. Both offer a presumption in favor of the conversion, but neither can stand in the way of positive evidence to the contrary.

On the other hand, there exist many facts which on the surface seem to indicate that, in the animal body, the conversion does not take place—the respiratory quotient considered as an indication of combustion, the D/N ratios as obtained by various workers, etc.,—facts which will be considered in their place. To many physiologists, these facts seem to offer conclusive proof against the conversion. To explain them away, certain assumptions must be made, the validity of which is at present a controversial matter.

For the sake of simplicity, it can be said that in order to believe in the obligatory conversion of fat to carbohydrate if the former is to be used as fuel, two major conceptions must be introduced: 1, that carbohydrate is the sole fuel for muscular exercise; 2, that in the completely diabetic organism there is no failure of the mechanism for oxidizing sugar.

I. The Fuel of Exercise. If carbohydrate is the sole fuel of exercise it follows that fat, to be used for this purpose, must first be changed to sugar. Upon what does the conception of the exclusive combustion of sugar rest, and is it valid? First of all, it is based upon evidence obtained in the isolated muscle.

A. The isolated muscle. 1. The utilization of fat. Leathes (1906),

tetanizing the muscles of one side of an animal and comparing them with the other side could find no evidence for a consumption of fat on the tetanized side. Winfield (1915) made a similar observation, finding that normal muscles and those fatigued anaerobically with aerobic recovery had practically the same content of fatty acids. On the other hand, Palazzolo (1912) found in frogs a reduction in the fat content of stimulated muscles as compared with unstimulated ones. In the hedgehog, this was even more marked, the fat content of the stimulated side being reduced one-half. These experiments are quoted because some stress has been laid on them. Nevertheless, the experiments of Palazzolo, even if sound, do not prove that the fat was directly oxidized instead of being converted to sugar; neither do the negative experiments of Leathes and Winfield prove that fat cannot be oxidized to provide the energy for muscular contraction. For, assuming the facts to be as stated by the last two investigators, it can, at most, only be said that fat must undergo some change outside of the muscles before it can be used. This might be a conversion to carbohydrate in the liver, as has been postulated; it might, on the other hand, be a preparation for direct oxidation, as for example, desaturation in the liver. Both of these are postulates—the evidence for the first will be considered later—but the above experiments offer no presumption in either direction. That under certain circumstances muscle can use fat coming to it is indicated by the experiments of Lafon, who observed an increase in the fat removed from the blood circulating through muscles when they were stimulated to contraction, compared with that noted when they were at rest. It is to be admitted, however, that this unsupported (though uncontradicted) experiment cannot be regarded as conclusive.

2. The R.Q. Very few observations exist on the R.Q. of isolated skeletal muscle. Meyerhof (1919) in developing the theory involving the oxidation of lactic acid, determined the respiratory quotient of minced frog muscle and found it to be in the neighborhood of unity. This observation has been used as an argument for the obligatory combustion of carbohydrate by voluntary muscle. Meyerhof and Himwich (1924) using the diaphragm of a rat, found the R.Q.s were not dependent on the previous diet, and tended to be high (av. 0.95). Takane (1926), working in Meyerhof's laboratory and also using the rat diaphragm, found that the consumption of fuel by the tissue, as measured by the respiratory exchange, exceeded that which could be provided by carbohydrate and protein, and concluded that fat must have been oxidized. If the muscle were conceded the ability to synthesize carbohydrate from

fat, this, of course, would not be conclusive, as some of the oxidized glycogen might have been replaced in that manner. No evidence exists to prove that muscle possesses such an ability. In experiments performed by Himwich and Castle (1927) the average R.Q. of resting muscle was similar to that obtained from the whole animal. The R.Q. of the muscles was arrived at by CO_2 and O_2 determinations of simultaneously obtained samples of the arterial and venous side of the muscle circulation. In view of the possible sources of error of such determinations of the R.Q.,——as, for example, the variations in blood flow and pressure, the question whether simultaneous samples of arterial and venous blood actually represent simultaneous consumption of O_2 and production of CO_2 by combustion in the tissue, the variable CO_2 and lactic acid content of the tissues, etc.,——it is not surprising that the mean error of the R.Q. in the experiments was 0.16. Such an error would mean that a respiratory quotient of 0.80, for example, might be either 0.64 or 0.96. In the later experiments of Himwich and Rose (1929), the deviation was less, the authors concluding that contracting muscles had the same R.Q. as that of the entire resting animal. But here again wide variations (0.67 to 0.94) were observed in the muscle R.Q.s under the same conditions of nutrition, consequently reservations must be made in their acceptance. A similar criticism can be made in regard to the experiments of Doisy and Beckman (1922), in which the average R.Q. of the hind leg muscles of the dog was 0.80. This much can be said, however, that apart from the single group of observations by Meyerhof, there is no evidence that even in the isolated muscle only carbohydrate can be oxidized. In the muscle removed from the body, it may well be that under certain circumstances respiratory quotients indicating the combustion of carbohydrate can be obtained. Assuming these to be true combustion quotients, they do not prove that in the intact animal only carbohydrate can be used as fuel for muscular contraction.

As far as isolated muscle itself is concerned, the view that carbohydrate is the only fuel that it can use is chiefly the result of the brilliant series of researches beginning with the experiments of Fletcher and Hopkins in 1911 and continued by Hill, Meyerhof, and their collaborators. These investigations emphasized the importance of carbohydrate in muscular metabolism. In the anaerobic phase, the conversion of glycogen to lactic acid was shown to play a predominant rôle. In the recovery phase, lactic acid is converted to glycogen, and since the heat produced in this phase was sufficient to account for only a small part of the lactic acid which actually disappeared, it was assumed (forti-

fied by the above mentioned observations of Meyerhof on the R.Q. of frog's muscle) that the oxidation of this moiety of the lactic acid provided the energy for the endothermic conversion of the remainder of the lactic acid to glycogen.

The emphasis that these researches placed upon carbohydrate was inevitable; nevertheless, from this type of work, there issues no certain proof that the oxidative phase of muscular metabolism is exclusively of a carbohydrate character. The initial phase of contraction is non-oxidative; in regard to the recovery phase, the thermodynamic data do not exclude the oxidation of fat. Needless to say, this is not the whole story, and we turn next to evidence obtained from the intact animal.

b. The respiratory quotient of the exercising animal. In view of the vital importance of this point in the discussion, a resumé of the evidence is advisable. The earliest pertinent evidence was offered by Chauveau (1896), who reported in brief form an experiment on Tissot, in which the resting R.Q. of 0.75 rose to 0.95 after forty-five minutes of running up and down stairs. After an hour's rest the R.Q. had returned to the basal level. Upon this experiment Chauveau formulated the theory that muscular exercise was accomplished at the expense of carbohydrate, and that fat, when used, was first changed to sugar. This, however, cannot be accepted as valid evidence in view of the fact that the total period of recovery was not studied, a period which the researches of Hill have shown to be an integral part of exercise. The high quotients during the exercise, as later work has indicated, may have been due to a "blowing off" of CO_2, compensated for by low quotients during recovery. In later communications, Chauveau (1897, 1898) buttressed his earlier conclusions by experiments in which there was apparently greater energy loss when fat was used in exercise than when carbohydrate was derived from preformed stores. But this was deduced from the fact that in dogs, having a constant caloric intake and doing the same amount of work, the loss of body weight was greater on a fat-protein than on a carbohydrate-protein diet. It has been frequently shown that on a fat diet the body holds much less water than on a regimen of carbohydrate, consequently, as Zuntz (1898) pointed out, the argument based on body weight was worthless.

Zuntz in a long series of researches by himself and his collaborators, beginning shortly after Chauveau's work, and summarized by him in a paper in Oppenheimer's Handbuch (1911), upheld the view that not only carbohydrate, but also fat could be oxidized in exercise. This

was based partly on experiments showing that the respiratory quotient of exercise was as much a function of the previous diet as was the resting quotient. For example, in experiments by Frentzel and Reach (1901), Zuntz's pupils, the average exercise quotient of Frentzel, running on a treadmill during a week in which he was on a high-fat diet, was 0.77; on a carbohydrate diet it was 0.89. The exercise R.Q. of Reach was 0.78 on the fat diet and 0.90 on the carbohydrate diet. From such experiments, as well as certain observations on the efficiency of exercise, which will be discussed later, Zuntz and his school concluded that carbohydrate was not the sole fuel of exercise.

Benedict and Cathcart (1913) also observed a change in the R.Q. of exercise according to the character of the diet; the average quotient on a carbohydrate rich diet, for example, being 0.88, on a carbohydrate-poor diet being 0.77. These investigators noted a rise in the quotient during work, but also a drop below the resting level after the work was completed, and it is difficult to say, from their data, whether the total period of exercise and recovery would have shown a rise in the quotient or not.

In the research of Anderson and Lusk (1917), a starving dog showed, during long continued exercise on a treadmill, respiratory quotients which were practically theoretical for the combustion of fat (0.71 to 0.73).

Krogh and Lindhard (1920), in an important paper which will be referred to again in the discussion of efficiency, studied the gaseous exchange in human subjects working on a bicycle ergometer while subsisting on high carbohydrate and high fat diets for protracted periods. These authors found that in a general way the R.Q. of exercise was a function of the diet, but that when the resting R.Q. was low, the quotient of exercise tended to rise somewhat; when the resting R.Q. was high, the reverse occurred. On the basis of this and their observations on efficiency, they *tentatively* suggested a conversion of fat to sugar when the respiratory quotient was below 0.80, and a conversion of carbohydrate to fat when it was above this figure. These well executed experiments do not prove that such conversions are actually taking place, and the investigators themselves carefully refrained from dogmatic assertions in regard to them.

Henderson and Haggard (1925) performed experiments upon well-trained oarsmen working to the limit of their capacity, and found that the quotients obtained during rest were very similar to those obtained when the individual was exercising violently. The average was 0.83 at rest and 0.82 during exercise and recovery. Meyerhof and Himwich

(1924), using rats that had been fed with excessive amounts of fat, found that during a period of work on a treadmill, the R.Q. was 0.7. This might have been due to the exclusive oxidation of fat, or to conversion of the fat (in the presence of a plethora) to sugar, with the immediate oxidation of the latter.

Certain of the earlier experiments mentioned above could be criticized in that they did not obtain the full gaseous exchange of recovery as well as of the exercise itself. But A. V. Hill and his school, in addition to the above cogent objection, criticized the earlier work on the ground that it was either long continued or very violent. In a series of researches (Hill, Long and Lupton, 1924; Furusawa, 1925; Furusawa, Hill, Long and Lupton, 1926) using the technique of analyzing samples of respired air obtained in Douglas bags, it was shown that in mild exercise of short duration, exercise and recovery quotients in the neighborhood of unity were always obtained no matter what the previous diet had been. As the exercise became more violent, or was prolonged, the respiratory quotient tended to fall. It was concluded that carbohydrate was the only substance oxidized in muscular exercise; that in mild exercise of short duration this carbohydrate was obtained from existing carbohydrate stores; and that as exercise was prolonged or made more violent fat was drawn on to supply the needed sugar, with a resultant fall in the R.Q. This could be harmonized with most of the findings of previous investigators.

The question seemed to hinge, therefore, upon the respiratory quotients of mild exercise of short duration. Rapport and Ralli (1928, 1) performed experiments in which an attempt was made not to exceed the severity or duration of the exercise in which R.Q.s in the neighborhood of unity had been obtained by the English investigators. The experiments were performed on dogs which ran on a treadmill that was introduced into a closed system of the Benedict-Homans type, permitting the total gaseous exchange of exercise and recovery to be obtained, as well as that of the resting period before and after. The exercise involved only a three- to four-fold increase in O_2 consumption. Under these conditions it was found that there was a close correspondence between the respiratory quotients of exercise and recovery, and the resting quotients, whether these were high or low, as will be seen in table 14. It was concluded that even in mild exercise the animal oxidized practically the same percentages of fat and carbohydrate as at rest, and that muscles, like other tissues in the body, oxidized carbohydrate, or fat, or both, depending upon the proportions in which these substances were presented to them in available form.

Marsh (1928), using human subjects doing moderate work, found that the respiratory quotient of exercise is a function of the previous diet. The precautions incidental to obtaining the recovery gaseous exchange were observed, and this investigator found that on a carbohydrate diet, the R.Q. of exercise was 0.95 to 1.00, while on a fat diet it was 0.80 to 0.83.

Wilson, Levine, Rivkin and Berliner (1928) have also observed in children a close correspondence between the R.Q. of exercise and that of the previous rest period.

TABLE 14

Comparison of the percentage of carbohydrate oxidized in the resting metabolism and in the excess metabolism of exercise

	RESTING METABOLISM		EXCESS METABOLISM OF EXERCISE	
	R.Q.	Carbohydrate per cent	R.Q.	Carbohydrate per cent
45-minute exercise:				
"Normal" diet	0.858	51.5	0.887	61.2
High carbohydrate diet	0.884	60.4	0.911	69.6
High fat diet	0.728	7.2	0.745	13.0
Average	0.823	39.6	0.848	47.8
15-minute exercise:				
"Normal" diet	0.904	67.3	0.869	55.3
High carbohydrate diet	1.012	100.0	1.017	100.0
High fat diet	0.744	12.7	0.751	15.0
Average*	0.860	52.2	0.854	50.2
Average of both exercises	0.846	47.5	0.852	49.5

* Includes experiment on a "moderately high" fat diet, and experiment in which fat was given two hours before exercise.

For mild exercise, the observation has recently been confirmed by Best, Furusawa, and Ridout (1929) and by Rapport (1929).

In the work of Best, Furusawa, and Ridout, however, as well as in that of Bock, Van Caulaert, Dill, Folling and Hurxthal (1928) a new conception has been introduced, in some respects at variance with previous ideas. Both of these groups of experimenters used human subjects, and studied the gaseous exchange by the Douglas bag method. In the work of Best, Furusawa and Ridout it was found that, although in mild exercise the quotient was practically that of the resting metabo-

lism, in severe exercise the R.Q. was unity or above.[2] In one case the quotient reached 1.7. A similar tendency for the quotient to rise with the severity of the exercise is to be seen in the experiments of Bock and his collaborators, this tendency being less marked in the better trained subjects. The authors conclude that carbohydrate is the fuel of exercise, but that with mild work the carbohydrate is obtained from fat, while in more severe work this process does not suffice, so that the stores of preformed carbohydrate are drawn on, with a resulting elevation of the quotient. Best, Furusawa, and Ridout come to a similar conclusion, though it is difficult to see how this could explain the very high quotients that they obtained. In their experiments they obtained samples of air throughout the exercise and recovery period. Bock and his co-workers, on the other hand, did not, assuming that a "steady state" existed on the basis of the constant total ventilation, steady pulse and respiration, and constant blood CO_2 and lactic acid. As to the existence of a "steady state," in so far as this relates to CO_2, it may be questioned whether the blood reflects so well the changes in CO_2, lactic acid, etc., of the other tissues that the "steady state" of the entire body can be safely assumed from it.

The results of these two groups of investigators are opposed to those of previous workers, for as we have already seen, the latter indicated that increasing the severity or duration of work did not have the effect of raising the exercise R.Q. A further elucidation of this contradiction would be desirable.

It is the reviewer's opinion that the evidence from the R.Q. does not justify the belief that carbohydrate alone is oxidized to supply the fuel for muscular exercise.

C. Thermodynamics. 1. The efficiency of exercise on different diets. Another argument for the obligatory conversion of fat, when used in muscular contraction, to carbohydrate, is based upon the differences of the efficiency with which exercise is performed upon diets in which one or the other of these foodstuffs predominate. It is assumed, when this contention is advanced, that the conversion of fat to sugar involves an "energy loss," and that this loss is reflected in the greater amount of energy necessary to do a given amount of work, other factors being the same, when the individual is working at the expense of fat rather

[2] It may be argued, as for example by Cathcart and Markowitz (1927), that the respiratory quotient cannot properly be regarded as a criterion of the character of combustion. Should this be so, it would cast as great doubt, however, upon the validity of quotients of unity as upon all other quotients.

than of glucose. In the first place, is there in fact such a difference of efficiency?

It has already been mentioned above that Chauveau (1897, 1898) deduced that fat was less efficient as a source of fuel for work than carbohydrate. Chauveau used this to support his earlier conclusion that sugar alone was used directly, and that fat needed first to be converted to sugar before it could be made available for the muscles. But, as we have seen, these deductions of Chauveau, being based on the criterion of body weight, have no value.

In Zuntz's laboratory, Heinemann (1901) was unable, in human beings, to observe any appreciable difference in the efficiency of exercise on fat and on carbohydrate diets.

Frentzel and Reach (1901), whose work on themselves has already been mentioned, found that when they were subsisting on a carbohydrate diet, they performed work slightly more economically than when they were living on a diet consisting chiefly of fat. Their data are as follows:

Frentzel	Fat Diet	= 2.07 Cals. per kilogrammeter
	CH Diet	= 1.98 Cals. per kilogrammeter
Reach	Fat Diet	= 2.12 Cals. per kilogrammeter
	CH Diet	= 2.09 Cals. per kilogrammeter

In neither case was the respiratory quotient indicative of the combustion of pure fat or pure carbohydrate, respectively. Krogh and Lindhard (1920) have calculated from these figures that the difference in efficiency as between fat and carbohydrate (that is, as between quotients of 0.71 and 1.00) would have been 7 to 11 per cent.

Atwater and Benedict (1903) obtained somewhat discordant results in human subjects working on a bicycle ergometer, the metabolism being determined in a closed respiration system. In many experiments there was no constant or considerable difference in efficiency on the two different types of diet. In the experiments on one subject, where the results were more directly comparable than in the others, carbohydrate seemed to be slightly more efficient than fat. Continuing these investigations, Benedict and Milner (1907) found that on the whole, exercise was more efficiently performed (about 11 per cent) on a carbohydrate than on a fat diet. In a later series of investigations from the Carnegie Nutrition Laboratory, Benedict and Cathcart found practically no difference in efficiency; for example, in one experiment 5.37 cals. per cal. of external work were required on a carbohydrate diet, and 5.31 cals. on a fat diet; in another, 6.66 cals. as compared with 6.68 cals.

Anderson and Lusk (1917), in their experiments on a dog, obtained the following results:

	Kgm. of energy per kgm.-m. of "work"
Starvation	0.578
Starvation	0.582
After 100 grams of glucose	0.550
Starvation	0.587

In other words, after glucose, work was done about 6 per cent more efficiently than on the predominantly fatty fuel of starvation.

Krogh and Lindhard, in their paper reported in 1920, to which reference has been made, made a careful and exhaustive study of the efficiency with which their human subjects performed work on different diets. They came to the conclusion that on the carbohydrate diets exercise was done at the expense of about 11 per cent less energy than on the fat diets.

The evidence, on the whole, appears to warrant the conclusion that muscular work is done somewhat more economically at the expense of sugar than at that of fat. But does it follow that fat, in order to be used, must first be changed to carbohydrate?

It is at least debatable whether, on other grounds, the exclusive combustion of carbohydrate by muscle has been demonstrated. Unless this postulate were accepted as proved, the question arises whether the studies on efficiency offer a compelling reason for accepting the belief. This should be decided on its own merits. The issue then becomes: do the thermodynamic data imply complete specificity or do they indicate at least partial non-specificity of the oxidative recovery process? Upon this, in turn, depends one phase of the question of the obligatory conversion of fat to sugar.

Certain experiments on the specific dynamic action of the foodstuffs have some bearing on this question. It has long been known that work is done uneconomically after the ingestion of protein, for the specific dynamic action of this foodstuff is summated with the extra heat resulting from work. Anderson and Lusk had found, as has been seen above, that this was not true for glucose. Rapport(1929) investigated the possible utilization in exercise of the "waste heat" of specific dynamic action, with the results shown in table 11. It will be seen that the specific dynamic action not only of glucose, but also of fat, is practically abolished; whereas that of protein is summated with the energy of work (tyrosin and epinephrin behaved like protein). Not

only could work be done at the expense of both fat and carbohydrate, but it appeared that the "waste heat" of their metabolism could also be utilized for this purpose. These experiments offer additional evidence in regard to the non-specificity of the oxidative reactions in muscle.

But apart from this, the question arises as to the validity of inferring chemical reactions from thermodynamic data under certain circumstances. This question should be examined, as it has a direct bearing on the matter at hand.

2. Theoretical. In the section on the conversion of carbohydrate to fat, the thermodynamics of the conversion, which would apply equally to the reverse reaction, was briefly discussed. In concluding that his

TABLE 15

Heat production at rest and in exercise and recovery

	RESTING (PER HOUR)	DEVIATION FROM (A)	EXCESS IN EXERCISE AND RECOVERY (PER KGM. OF WORK)*	DEVIATION FROM (A)
	Calories	*per cent*	*Calories*	*per cent*
A. In the post-absorptive state	12.7		2.40	
B. After ingestion of fat	15.2	+19.7	2.54	+5.8
C. After ingestion of glucose	15.4	+21.3	2.38	−0.8
D. After ingestion of meat:				
100 grams	16.2	+27.6	3.38	+40.8
200 grams	17.8	+40.1	3.48	+45.0

* Based on ½ hour period after beginning of exercise, for reasons discussed in the text.

data on efficiency presented an argument in favor of the conversion of fat to sugar, Chauveau postulated the following reaction:

$$2\ C_{57}H_{110}O_6 + 67\ O_2 = 16\ C_6H_{12}O_6.$$

This reaction is about 30 per cent exothermic, and as Chauveau's loss of efficiency when fat was used approached this figure, he concluded that the decreased efficiency was due to the conversion. Zuntz and his school assumed a somewhat different reaction:

$$2\ C_{57}H_{110}O_6 + 49\ O_2 = 19\ C_6H_{12}O_6.$$

This is about 25 per cent exothermic, and since the results obtained by Zuntz and his collaborators showed differences of efficiency on the two

diets that were considerably less than this, Zuntz concluded that the reaction did not take place. We have already seen that in other investigations, notably the carefully executed researches of Anderson and Lusk, and of Krogh and Lindhard, the loss of efficiency on a fat diet was not above about ten per cent. This would bring the results theoretically in harmony quantitatively with certain other formulae, for example:

Hanriot	$C_{55}H_{104}O_6 + 72\ O_2 = 13\ C_6H_{12}O_6$
Magnus-Levy	$2C_{55}H_{104}O_6 + 155.5\ O_2 = 27\ C_6H_{12}O_6$
Gauthier	$C_{57}H_{110}O_6 + 75\ O_2 = 14\ C_6H_{12}O_6$

These reactions involve respectively a 7 per cent, 11 per cent and 11 per cent exchange of energy. They are, however, endothermic.

The theoretical energy loss in the conversion has on many occasions been used as an argument in favor of its occurrence, as for instance in the recent monograph of Macleod (1928), this author using the Zuntz formula for the purpose. The oxidation in the recovery process of muscular contraction of a certain percentage of the lactic acid formed in the initial phase has been similarly inferred, in part, from thermodynamic data; and, as we have seen (section on protein → carbohydrate) theories of the specific dynamic action of protein, involving definite reactions have likewise been postulated in this manner.

Krogh and Lindhard have, in the present connection, rightly called attention to the danger of assuming specific chemical reactions on the basis of the thermodynamics of bodily processes, and suggest that a formula could very well be written for the conversion of 1 mol. of fat to 12 mols. of glucose that would be eighteen per cent endothermic.

The terms "energy loss" and "energy gain" are frequently used in the discussions of this subject, and sometimes lead to confusion. Taking a given reaction as a closed thermodynamic system, it is clear that if the reaction is exothermic, a "loss" of energy from the system has taken place; if it is endothermic, a "gain" of energy results. But actually, the reaction, when we are studying the efficiency of the whole organism, can not be considered as a closed system.

If the reaction happens to be endothermic, the sum total of reactions in the body associated with it may result in a "loss of energy" to the body, although the reaction itself may represent a "gain." For example, suppose one makes the assumption that fat is endothermically converted to sugar and the latter oxidized. It is possible to argue that the extra energy is "lost" to the body as a result of coupled exothermic reactions

which not only supply the energy for fat → sugar, but an extra amount which is not available as free energy for the body. Such a theory has been advanced to explain specific dynamic action. It is quite within the bounds of possibility and the theory might equally apply to the present case.

But if we assume on the contrary that the conversion of fat to sugar is exothermic, energy "loss" to the body can equally well be postulated. In both cases, whether a "loss" of energy will occur will depend upon whether the energy released by *all* of the reactions involved can be utilized as free energy, and not upon the thermodynamic character of the single reaction.

Two difficulties are present therefore in this type of reasoning. In the first place, unless one knows the specific chemical reactions, it is impossible to say whether a conversion of this character is exothermic or endothermic. In the second place, even if one knows this, one still cannot say that a given energy "loss," as observed in efficiency experiments, is due to a given reaction, unless one knows how much, if any, of the released energy can be utilized for specific purposes. Thus the specific dynamic action of protein always, apparently, appears as "waste heat," in other words is "lost." But that of carbohydrate and fat can be used in muscular exercise, and under these conditions is not lost. Exothermic reactions seem then to differ in their utilizability, and until we know more about the conditions of their utilization, speculations appear fruitless. The investigation of this general field is a major problem of metabolism.

On the basis of the above, it seems to me that the data on the difference in efficiency of muscular exercise on carbohydrate and fat diets cannot justifiably be used as an argument in favor of the conversion.

In regard to the whole question of the use of carbohydrate for oxidation in muscular contraction, at best it can only be said that the case for its exclusive use is not proved. It is the reviewer's opinion that the evidence favors the view that, to provide energy for muscular activity, fat can be directly oxidized as well as carbohydrate, and that, from this particular kind of evidence, the conversion of fat to sugar cannot be inferred.

II. The Theory of Diabetes. While physiologists as a rule believe that the primary disturbance in diabetes is the partial or complete failure of the organism to burn sugar, there are not wanting distinguished investigators who are of the opinion that the sugar oxidative

mechanism is unimpaired. These believe that in diabetes there is an overproduction of sugar from protein and fat, and that such failure of oxidation as occurs is due to the fact that more sugar is thus synthesized than can (or needs to) be oxidized. As will be seen later, this theory of diabetes follows logically from a belief in the conversion of fat to carbohydrate.

a. The R.Q. in diabetes. 1. In starvation. In the section on the conversion of protein to carbohydrate, allusion was made to the diabetic respiratory quotient for the purpose of seeing whether this was compatible with the origin of sugar from protein, which it clearly is. It has been questioned, however, whether the lowest quotients obtained do not necessarily imply the formation of sugar from fat as well. Pembrey (1901) calculated that the R.Q. of the conversion was 0.28, and it can be further calculated that any probable conversion from mixtures of fats or fatty acids to carbohydrate would have a respiratory quotient not far from this figure. No authentic R.Q.s in this neighborhood have been obtained, and in the various forms of diabetes it is rarely below 0.66 to 0.70. Nevertheless the conversion could be inferred as a possibility, at least, provided the obtained quotients were lower than could be accounted for by production of sugar from protein. (This does not take into account the effect of failure to oxidize the ketone bodies or other intermediary metabolites.)

We have already seen in the section on protein → carbohydrate that in the starving and resting human diabetic quotients in the neighborhood of 0.66–0.70 are the rule in the most severe cases, although in single instances apparently authentic R.Q.s as low as 0.61 have been obtained. A certain amount of difficulty is inherent in the interpretation of the human respiratory quotients, chiefly on account of the fact that it is hard to control the experimental conditions. In the exhaustive studies of Benedict and Joslin (1910, 1912) on severe diabetics, the starving R.Q.'s were between 0.69 and 0.76. In the case observed by Geyelin and Dubois (1916), where the D/N ratio was as high as 3.97/1, the average R.Q. was 0.69; and in the case studied by Wilder, Boothby and Beeler (1922), the quotient was 0.70 when the D/N was in the neighborhood of Lusk's ratio. In the fully phlorhizinized animal, as has been stated in another section, the fasting quotients are practically never below 0.69, and may be as high as 0.71. In the depancreatized dog, the range is usually between 0.67 and 0.74, as will be seen from the following illustrations:

Lafon (1906)	0.71–0.73
Mohr (1907)	0.64–0.73
Falta, Grote and Staehelin (1907)	0.68–0.72
Verzár and Fejer (1913)	usually 0.70 or above (one of 0.62)
Verzar (1914)	0.70–0.73
Moorhouse, Patterson and Stephenson (1915)	0.67–0.74
Hédon (1926)	0.70 or above*
Chaikoff and Weber (1928)	0.67–0.70
Chaikoff and Macleod (1929)	0.68–0.70

* Except immediately after withdrawal of insulin.

These results, presenting a fair cross section of the literature, show only isolated instances of starving quotients below 0.67.

Taking the three types of diabetes, it seems that on the whole there is a tendency for the fasting respiratory quotient to drop slightly below the normal fasting quotient. Do these quotients indicate the formation of sugar from fat? Magnus-Levy (1904) calculated theoretical protein quotients that might be obtained in the diabetic. According to this author, they might be as low as 0.61. Lusk (1915) has calculated that with a D/N ratio of 3.65/1, the diabetic R.Q. of protein would be 0.632 as follows:

	O_2 *grams*	CO_2 *grams*
Normal oxidation of 100 gm. beef protein	138.18	152.17
Deduction for 3.65 x 16.28 gm. N (Corresponding to 59.41 gm. glucose)	63.38	87.15
	74.80	65.02

With these values for the R.Q. of protein, assuming no sugar to be oxidized, the quotients actually obtained in the diabetic state are easily explainable, except in rare instances which cannot, in view of the known difficulty of obtaining absolutely accurate quotients, be permitted to invalidate the weight of evidence.

If the amount of sugar obtainable from meat protein should correspond to the Minkowski ratio of 2.8 to 1, rather than to that of 3.6/1, the diabetic quotients would be somewhat less easy of explanation on the basis that sugar was not obtainable from fatty acids. Lusk (1915) has calculated the diabetic R.Q. of the sugar-forming acids of meat protein to be 0.675, from which that of the non sugar-forming acids could be calculated as 0.716 (or according to a direct calculation 0.76).

These figures imply a smaller formation of sugar from meat protein than the 59 per cent implied in the D/N ratio of 3.65/1.

The following calculation can be made of the yield of glucose from the known sugar-forming amino acids of meat protein (table 16).

This yield of glucose would mean that approximately forty-four per cent glucose is obtainable from protein, and would correspond practically to the Minkowski ratio.

A further calculation (table 17) can be made, using data similar to those in table 16, of the diabetic R.Q. of meat protein assuming 39 mols of glucose to be obtainable from the molecule of protein. Such a calculation is of course hypothetical, but if sound, the respiratory quotient of the diabetic animal when fasting would be above that of fat, provided no sugar were formed from fat.

TABLE 16

Glucose from meat protein

AMINO ACID	PER CENT BY WEIGHT IN PROTEIN MOLECULE	PROBABLE NUMBER OF MOLS	CARBON PER MOL	TOTAL CARBON
Glycine	4.0	9	2	18
Alanine	8.1	14	3	42
Aspartic acid	10.6	10	3	30
Glutamic acid	22.3	24	3	72
Proline	8.0	11	3	24
Arginine	11.5	3	6	33
Cystine		3	6	18

39 mols. of glucose = 237.

But even if we assume the Minkowski ratio and the above calculations, quotients which do not fall much below the theoretical value for fat are still explicable in complete diabetes, owing to the imperfect oxidation of fats. Unfortunately, it is difficult to quantitate this factor, for while the failure to oxidize the ketone bodies would tend to lower the R.Q., the blown-off CO_2 resulting from their accumulation in the body would tend to raise it. Magnus-Levy (1904) has calculated the possible result, but in a given case the effect would be largely problematical. It is perhaps sufficient to say that such fasting quotients in diabetes as are actually obtained do not necessarily invite the presumption of a conversion of fat to sugar.

2. After food. The R.Q.s after the ingestion of food also do not involve the necessity of accepting this view. The ingestion of sugar does not raise the respiratory quotient in complete diabetes (e.g., Bernstein and Falta (1918), Wilder, Boothby and Beeler (1922), Geyelin and Dubois (1916), in human diabetics; Verzár and Fejer (1913), Verzár (1914), Campbell and Markowitz (1927) in depancreatized animals). According to the protagonists of the conversion of fat to sugar, this simply means that in an animal in which sugar is already being produced in excessive amounts from protein and fat, the addition of still

TABLE 17

Theoretical diabetic R.Q. for meat protein

	C	H	O	N	S
Sugar-forming amino acids	359	718	238	118	6
Glucose, 39 mols	234	468	234		
Non-sugar-forming amino acids	125	250	4		
	284	524	90	62	
Urea (90 mols)	409	774	94	180	
	90	360	90	180	
6 SO_3	319	414	4		
			18		6
			−14		
To oxidize hydrogen			207		
To oxidize carbon			638		
			859 = 429.5 O_2		

Deduct CO_2 for Formation of urea from 10 Mols NH_3 = 319 − 5 = 314 CO_2

$$R._2 = \frac{314}{429.5} = 0.731$$

more sugar cannot be oxidized even by normal oxidative mechanisms (Macleod, 1928). In other words, the situation would in a sense be not unlike a normal alimentary glycosuria, though without the irregularity of response characteristic of the latter. Any failure of sugar to be oxidized would fall into this category.

This theory would account also for the effect of ingesting protein. The normal R.Q. of protein, which is above 0.80, is dependent upon the

oxidation of all of the non-nitrogenous portion of the molecule. I have already alluded to the theoretical diabetic quotients of this foodstuff. Various observers have incidentally administered protein to diabetics, examples of the resulting R.Q.s being as follows: Benedict and Joslin (1910) 0.71–0.74; Wilder, Boothby and Beeler (1922) 0.64–0.66. These experiments were on human diabetics, and it is of interest that in the experiments of Wilder, Boothby and Beeler, there was a fall of the quotient when protein was given. If this was a protein effect, it would seem to bear out the theoretical diabetic quotients of this material advanced by Lusk and Magnus-Levy. (The ingestion of carbohydrate, however, has also been noted occasionally to produce a fall in the quotient.) In depancreatized dogs, Hédon (1926) has observed quotients of 0.72 to 0.75 on giving 100 and 200 grams of meat. In no case where it was clear that the animal was completely diabetic have R.Q.'s been obtained high enough to indicate the combustion of the carbohydrate portion of the protein molecule.

But if it is argued that again this is similar to the situation when sugar is ingested, namely, the overtaxing of a normal oxidative mechanism, it is difficult to see how this can be reconciled with the effects of fat ingestion. It is generally admitted that no striking change occurs in the respiratory quotient of the completely diabetic animal when fat is administered. It remains in the neighborhood of 0.69–0.70, no matter how much or how little fat is administered. Now if gluconeogenesis is occurring under these conditions (as would be inferred from the interpretation of the D/N ratio, for example, in fat as compared with lean animals—see Macleod, 1928), such sugar must in fact be almost entirely oxidized, else the R.Q. could not remain, as it actually does, in the neighborhood of the theoretical value for fat combustion. (The necessary synchronization of conversion and oxidation under these circumstances must be emphasized. The sugar deriving from fat must not only be oxidized but immediately oxidized. Storage of formed sugar becomes inadmissible.) In other words, the sugar obtained from fatty acids, according to this theory, must behave, under similar conditions in the body, in a manner entirely different both from pre-formed or ingested sugar and from that derived from protein, both in starvation and after food. Unless one makes the gratuitous assumption that fat ingestion depresses glyconeogenesis in a way that protein and sugar do not, such a conception is obscure and difficult of acceptance. Nevertheless it is not impossible, though it would appear to be inconsistent

with a theory that must explain low diabetic quotients on the ground that sugar derived from fat is at least partially *not* being oxidized.[3]

3. In exercise. The data on this subject are not extensive, although under the conditions of calorimeter experiments variations in the activity of the experimental diabetic subject must have frequently occurred. Grafe and Salomon (1922) found that when a diabetic with a resting R.Q. of 0.72 was made to exercise, the resulting quotient was 0.71. Richardson and Levine (1925) also failed to observe a rise in the respiratory quotient of exercising diabetics. In the experiments of Hetzel and Long (1926), the R.Q. of exercise in diabetics was 0.80 as compared with the resting R.Q. of 0.76. The slight change would not seem to warrant the authors' conclusion that fat was being converted to sugar in order to provide fuel for exercise. From the evidence it would appear that the metabolism in exercise was of a character similar to that at rest. This conclusion was drawn by Rapport and Ralli (1928, 2) who found that in the phlorhizinized dog there was no greater tendency for the oxidation of sugar in exercise than at rest. In phlorhizin diabetes, however, the power to oxidize sugar is not entirely lost, as has been shown by Wierzuchowski (1926, 1927) and by Deuel, Wilson and Milhorat (1927), as well as by the above mentioned experiments. The same applies to the human diabetics. Recently Chaikoff and Macleod (1929) have reported experiments in which the R.Q. of depancreatized dogs, whose resting quotients were between 0.68 and 0.70, rose during the first half-hour of shivering induced by a cold bath and exposure to cold in a respiration chamber. The excess R.Q. was as high as 1.05 during this period, though more often between 0.75 and 0.80. It was concluded that the rise was due to the oxidation of pre-formed carbohydrate, and that it was less than in the normal animal because the

[3] Since this paper went to press, a communication by Soskin (Biochem. Journ., 1929, xxiii, 1385) has appeared purporting to show extra sugar excretion in depancreatized dogs on feeding fat and lipase or lecithin. Ten of the 13 experiments were negative; in two experiments after lecithin, and in one after a mixture of olive oil and castor bean suspension, "extra" sugar appeared in the urine. The negative experiments are attributed to the "physiological limitations" of the method. With one exception, there was no lowering of the R.Q. seen in the "positive" experiments, such as might be expected if excreted sugar were formed from fatty acids. The exception occurs in Expt. 18, and the author regards the lowering of the R. Q. in this case as significant for "the support it lends to the conversion of fat to carbohydrate." The reviewer agrees that this result may be significant, but not for the reason given by the author, for the fat used in this experiment was "intarvin," an odd-carbon fat!

carbohydrate reserves were smaller in amount. The authors rule out depressed gluconeogenesis from fat as a factor because of the increased oxygen consumption.

This rise might be considered as constituting important evidence for the capacity of the totally diabetic organism to burn sugar, and consequently indirect evidence for the formation of sugar from fat. Against its acceptance are the results of the experiments quoted above. Moreover, the exercise involved is a mild one (the oxygen consumption in the period was about double the resting value), and in this type of exercise the evidence obtained from the normal animal favors the view that the excess quotient of exercise, when the recovery period is included, is practically the same as the resting R.Q. The later periods of shivering showed no rise in the quotient. The final recovery periods were not obtained, since the respiration chamber was kept at low temperature throughout the experiment. It is at least questionable whether, in the absence of data regarding such a recovery period, the rise of quotient during the first half-hour can be accepted as an index of the type of food metabolism. The blood CO_2 content of one animal was determined before and after a shivering period comparable with those in which the gaseous exchange was determined. No significant differences were observed, yet the control can hardly be regarded as conclusive, for reasons discussed in the section on the R.Q. of exercise in the normal animal. However, the evidence presented by these experimenters is not to be dismissed lightly, and further investigation should be undertaken. In an experiment on a depancreatized dog by Ralli and Rapport (unpublished) the resting R.Q. of 0.71 was unchanged during mild exercise and recovery thereform.

b. The D/N ratio. There is almost complete unanimity that the ingestion of fat has no effect upon the excretion of sugar in diabetes. Von Mering (1888, 1889), noted that this was true not only in the phlorhizinized dog but in severe cases of diabetes mellitus, an observation confirmed for the phlorhizinized animal by Moritz and Prausnitz (1890), Rosenfeld (1893, 1912), Lusk (1901), Loewi (1902), Ringer and Lusk (1910), and Takao (1926). Kojima (1927) found that glycerol increased the sugar excretion in phlorhizinized rats, and in a later paper (1928) found that while sodium lactate and meat increased the sugar excretion, butter and lard increased it less than would be expected from the glycerol component of the fat. He concluded that fat actually hindered sugar formation. Fatty acids could not be tested, as they were not tolerated by the animals.

The only exception to the general agreement is in the experiments of Calvo-Criado (1925) who on giving fat to phlorhizinized rats concluded that extra sugar was being excreted. In these experiments there were wide fluctuations in the D/N ratio, and frequently on a pure meat diet the ratios were higher than after fat. Apparent rises the day after fat was given were often associated with a marked fall in the ratios on the day before, that is, the day on which fat was ingested. The influence of fat was variable, and the conclusions do not appear to be justified.

Geelmuyden (1920) concluded that the ingestion of oil gave rise to extra sugar in phlorhizinized rabbits, but the experiments hardly bear this out, and in 1923 Geelmuyden himself admitted that fat ingestion was without effect on the sugar excretion. In the same research (1920) he found that acetic and butyric acid caused increased sugar excretion; caproic acid, however, yielded negative results. These findings in respect to acetic acid have been denied by Deuel, Wilson and Milhorat (1927). Ringer and Lusk (1910) had also failed to obtain an extra sugar excretion from acetic acid.

The fact that fat does not increase the sugar excretion or the D/N ratios in diabetes, is explained by the proponents of the conversion of fat to sugar as indicating that the formed sugar is oxidized. The same objection applies here as in the case of the diabetic R.Q. for the D/N ratio is clearly affected by ingested sugar, and the excretion of sugar is markedly increased by the ingestion of protein, with no change in the D/N, as already shown in a previous section.

Examples of the D/N ratios obtained in the fasting diabetic animal have already been given in the section on the conversion of protein to carbohydrate. These may be summarized by saying that in the vast majority of cases ratios approximating either the 2.8/1 ratio of Minkowski or the 3.65/1 ratio of Lusk, have been obtained. There have been certain exceptions to this, and recently Macleod and his co-workers have questioned the validity of the ratio as an index of the sugar obtainable from protein, on account of the fluctuations they have observed in the ratios of diabetic animals.

Chaikoff, Macleod, Markowitz and Simpson (1925) reported experiments in which, after withdrawal of insulin in depancreatized dogs, D/N ratios considerably lower than the Minkowski ratio were observed, although it was approached in two of the five cases studied. In two others there is evidence that sugar was being utilized by the animal.

Chaikoff (1927) observed the following D/N ratios in depancreatized dogs:

Dog 1 (Fat)..... 3.3/1
Dog 1 (Thin)..... 2.88/1
Dog 2 (Fat)..... 5.5/1
Dog 2 (Thin)..... 3.12/1
Dog 3 (Fat)..... 4.3/1
Dog 4 (Thin)..... 3.13/1

In dog 4 the later ratios were low (2.0 and 2.4). It is important to note that none of the observations were made after the fourth day following withdrawal of insulin. A similar tendency for fat dogs to have high ratios was observed by Halsey (1899) in phlorhizinized animals.

TABLE 18

(Macleod, 1928)

G:N ratios of dogs depancreatized about two months previously

NUMBER	WEIGHT	DAYS AFTER REMOVAL OF INSULIN. NITROGEN IN GRAMS														AVERAGE BEYOND 2ND DAY
		II		III		IV		V		VI		VII		VIII		
		N	G:N	N	G:N	N	G:N	N	G:N	N	G:N	N	G:N	N	G:N	
	kgm.															
1	4.1			4.5	2.4	3.9	1.8	3.0	2.0	3.0	1.8	3.7	1.7			
2	7.3			2.6	8.8	3.8	2.8									
3	7.6			4.2	10.4	6.4	4.9	2.9	5.2							
4	5.9							2.7	3.3	3.8	2.4	4.3	3.1			
5	5.7							2.4	5.1	2.4	2.5	2.5	1.0			
6	6.3							3.9	3.6	4.8	3.2	4.9	2.5			
7	5.4							4.4	5.9	5.3	3.6	5.5	3.3			
8	3.9			2.3	18.1	2.9	3.9	3.4	2.5							
9	9.2	6.9	6.8	8.5	4.9	6.9	3.6	4.7	3.6	5.6	2.8	9.6	2.6	4.6	2.1	
10	4.5			4.6	2.6	5.0	2.7	6.5	2.0							

Macleod and Markowitz (1926) noted the D/N ratios in 10 dogs, the results being summarized in table 18. If the third day after withdrawal of insulin be excluded, the following are the average D/N ratios of the ten dogs: 1.8; 2.8; 5.1; 2.9; 2.9; 3.1; 4.3; 3.2; 2.9; 2.4; the grand average being 3.05. This is suspiciously close to the Minkowski ratio, and in only two of the animals is it exceeded to an appreciable extent. Macleod recognizes that averages may approach this ratio, but stresses individual variations. There can be no doubt that in certain cases high D/Ns are obtained, but may these exceptional cases not in part be due to exceptionally high residual glycogen stores? Moreover, the fact that fat (i.e., well-nourished) animals are the ones which show

the highest D/Ns may be due to the fact that these animals, being well nourished, have not only stored more fat but also more glycogen. It may be questioned whether in all cases the residual glycogen has been exhausted by the third day. Another factor, though possibly a minor one, should be considered, namely, the tendency for the nitrogen excretion to rise during the early days after pancreatectomy. This tendency is also to be observed immediately after withdrawal of insulin in Chaikoff's experiments (1927). At all events, a discrepancy in the speed of elimination between the N and sugar of protein might well play a part in accounting for daily variations.

The reviewer is of the opinion that owing to these considerations, the high D/Ns obtained in the above investigations need not be interpreted as invalidating the D/N ratios as an index of sugar derived from protein.

As to the low ratios, such as have been observed by Embden and Salomon (1905) in a very small dog, and in the above mentioned researches of Macleod and his collaborators, it is doubtful whether they can be used as an argument for the conversion of fat to sugar. If the amount of sugar relative to N appearing in the urine of diabetic animals is less than what is customarily observed, it may be attributed to an impairment of the sugar synthesizing mechanism, whether that synthesis be from protein or fat. It is perhaps significant that the D/N ratio tends to fall markedly on the day before death. At this time, also, there is often observed a high respiratory quotient, which is clearly not a function of increased sugar oxidation, but rather a phenomenon attributable to faulty metabolism.

In exercise, the D/N ratio of the fasting diabetic animal is unaffected (Lusk, 1908; Allard, 1908), in spite of the fact that the utilization of fat is greatly increased. To explain this in terms of the conversion of fat to sugar, it has to be assumed that the speed of the conversion is accelerated to just the degree required to provide sugar for fuel in addition to the customary "waste" sugar, in other words, to attribute a nicely exact regulatory function to a mechanism which has run amuck.

c. The blood sugar. This will be discussed in connection with liver function. Certain observations, however, are pertinent at this time. Csonka (1916) noted that in phlorhizinized dogs with the ureters ligated, ingested glucose and protein resulted in hyperglycemia; ingested fat, however, did not. Parnas and Wagner (1922) studied a young girl suffering from a liver tumor. On fasting, there was no measurable blood sugar. Ingested carbohydrate, protein, glycerol, and amino acids caused

a definite rise in the blood sugar, but feeding of olive oil in sufficient quantities to produce lipemia and lipuria did not. Pollack (1922) injected normal rabbits with various substances. Sugar-forming amino acids and saturated fatty acids with an uneven number of carbons produced hyperglycemia; even carbon chain fatty acids did not (see Ringer, 1912). Hanisch and Junkersdorf (1927) observed that the hypoglycemia of fasting phlorhizinized dogs could be prevented by a high protein diet, but not by a high-fat diet. These observations all tend to show that sugar is not formed from fat.

d. Isolated tissues (apart from skeletal muscle and liver). The experiments have been carried out chiefly on the heart. The results are not as a rule clean-cut, and the interpretation is difficult. Knowlton and Starling (1912), using the heart-lung preparation came to the conclusion that the heart of the depancreatized dog consumed much less sugar than that of the normal dog. This was based on the rate of sugar disappearance in the perfusing blood. Although the result was confirmed by Maclean and Smedley (1913), Starling, on the basis of a repetition with Patterson (1913) of the earlier experiments, abandoned his previous view. Macleod and Pearce (1913, 1914) attacked the validity of drawing conclusions from the rate of sugar disappearance, and in the light of present day knowledge regarding the variable glycogen content of tissues, the possibility of glycolysis via lactic acid, etc., their criticism appears justified. They themselves could find no difference in the rate of disappearance of sugar in the blood of normal and depancreatized animals. Clark (1916) found that the rate of disappearance of sugar from the perfusing blood of an isolated heart of the depancreatized dog was accelerated by a substance given off by the pancreas when blood is perfused through it—presumably insulin. Burn and Dale (1924) noted that in respect to the amount of sugar disappearing, the normal rabbit heart behaved like that of the diabetic animal. On the basis of the CO_2 production, these authors concluded that the "lost" glucose was burned in both cases. However, the CO_2 production in an isolated tissue such as this is an unreliable index of the rate of oxidation. (Visscher, 1928, found, on the basis of simultaneous studies of the O_2 consumption and sugar disappearance, that as much as $\frac{4}{5}$ of the total metabolism of the normal isolated heart may be non-carbohydrate, when carbohydrate is not abundant. No observations were made on the diabetic heart.)

In the experiments of Starling and Evans (1914) the average R.Q. of the normal heart, as determined from the CO_2 and O_2 content of the entering and leaving perfusing blood, was 0.85; that of the heart of

depancreatized animals, 0.71. In more recent experiments Peserico (1925) found the R.Q. of the isolated normal rabbit and cat heart to be higher than before, when insulin was added to the perfusing fluid. In the depancreatized cat the contrast was even more marked, the heart perfused without insulin having an R.Q. of 0.72; with insulin an R.Q. of 0.90. The $NaHCO_3$ content of the perfusate was noted as a control.

Nevertheless, in all of these experiments, too much reliance cannot be placed upon the respiratory quotients, particularly as to the numerator, for the fluctuations of the CO_2 content of the tissue remain to a large extent an uncontrolled variable. This is reflected in the wide variations of the R.Q.s obtained, for example, by Starling and Evans.

Verzár and Krauss (1914) noted that in the isolated gut of depancreatized dogs glucose disappeared as rapidly as in that of the normal animal, but themselves denied that sugar disappearance signifies oxidation. Richardson, Loebel and Shorr (1926, 1928) observed the R.Q. of excised rat kidney. In the normal animal the quotient varied from 0.71 to 0.91, depending upon the previous feeding of the animal; in the diabetic animal the quotients were in the neighborhood of the usual diabetic quotient for the entire animal, −0.69.

From the results quoted above, though they are on the surface somewhat contradictory, it seems fair to say there is no satisfactory support for the view that isolated tissues of totally diabetic animals can oxidize sugar, with the resulting implications as to the conversion of fat to carbohydrate.

e. The mechanism of insulin action. It is clearly beyond the scope of this paper to review extensively the nature of insulin action. This has been done by Macleod (1926), to whose monograph the reader is referred, and which has been used freely in the ensuing discussion, as well as to a recent paper by Grevenstuk and Laqueur (1925).

In the present connection, it is important chiefly to recall that the theory of diabetes involves either 1, a primary failure of the mechanism for oxidizing sugar, or 2, a primary overproduction of sugar from protein and fat (the two are not necessarily mutually exclusive, as was pointed out by Minkowski, 1906, and as has recently been emphasized by Grafe). If it is assumed that diabetes is due to the lack of insulin, then this hormone should correct primarily either 1 or 2, according to which theory is held. There is, so far as I am aware, no direct evidence that insulin depresses the formation of sugar from fat, as was held by Laufberger (1924).

As to the nature of insulin action, a few points may be briefly men-

tioned, in so far as they have a bearing on our problem, recognizing that frequently the mechanism is obscure.

1. The rise of R.Q. following insulin. This occurs in both normal and diabetic animals. *Per se*, it can be explained as due either to a facilitation of sugar oxidation directly or to an inhibition of sugar formation from fat. One's opinion must depend upon other phenomena.

2. The disappearance of ketonuria following insulin. This also is explicable according to both theories, though not completely explained by either. Nevertheless, it is not illogical to suppose, with Geelmuyden, that if the ketone bodies are intermediary products of the conversion of fat to sugar, they would tend to disappear as the process is inhibited. The alternative theory offers no satisfactory explanation of Rosenfeld's classical dictum at the present time.

3. The glucose equivalent of insulin. As administered glucose is increased, the amount accounted for per unit of insulin is also increased, though this is not a linear function (Macleod, p. 96 ff). This would appear to be inexplicable on the theory of inhibition, unless the increased amount of glucose in itself inhibited its formation from fat. But if the latter were true, the fact that glucose does not raise the respiratory quotient in complete diabetes would also become inexplicable.

4. Insulin causes injected glucose to disappear more rapidly than can be explained by inhibition of sugar formation (Macleod, p. 300 ff), though it is true that increased oxidation may not account for all of the disappeared sugar (Macleod, p. 170; 250, ff).

5. Insulin facilitates the deposition of glycogen in the diabetic animal, while at the same time causing a decrease in blood and liver fat (Macleod, p. 104; 163 ff). (This has indeed been used by Wertheimer (1926) to prove that insulin *facilitates* the conversion of fat to sugar, though it might equally well be explained on other grounds. For example, if one supposes that in the absence of carbohydrate oxidation the flooding of the blood and liver with fat is due to an excess reaction of the body to the need for fuel, a restoration of the capacity to oxidize sugar would tend to check this process.)

6. Insulin accelerates the hypoglycemia of hepatectomized animals. (Mann and Magath, 1923.) This is difficult of explanation on the inhibition theory, if it is assumed (see later discussion) that the formation of sugar from fat is primarily a function of the liver. The phenomenon occurs in eviscerated animals as well, according to Burn and Dale (1924), while Burn and Marks (1926) state that insulin has no effect on the production of sugar from non-protein sources in the liver.

7. Insulin increases carbohydrate oxidation at the expense of liver glycogen (Cori and Cori, 1928, 2). This would appear to be rightly interpreted by the authors as a direct effect on oxidative capacity, as it is inexplicable on the inhibition theory in view of 5 and 6.

The reviewer recognizes that this treatment of the effect of insulin is inadequate, and regrets that the exigencies of space render impossible a more extensive discussion. Nevertheless he feels that the above outlined considerations favor the view that insulin acts rather to facilitate directly the combustion of sugar, and not to inhibit the postulated overproduction of sugar from (protein and) fat. If this is true then in diabetes the fault lies primarily in the failure of the oxidative mechanism, and the conversion of fat to sugar lacks support on this ground.

III. The Rôle of the Liver. The tendency has been for those who favor the view that fat is converted to carbohydrate, to believe that this process is a function chiefly, if not entirely, of the liver. This would follow from their view that isolated tissues and particularly muscle, are unable to utilize fat directly. As we have already seen, the failure to utilize fat by excised muscle, even if sound, does not prove that fat must be converted to sugar, presumably in the liver, before it can be used. A preparation of the fats for oxidation, for example by desaturation, might account for the observed phenomenon, even if it were proved to occur. It is necessary, therefore, to examine the direct evidence for the conversion in the liver.

a. Glucose formation from fat. We may briefly mention a few of the older experiments with incubated liver. Seegen (1886) observed an increase in the liver sugar on digesting liver pulp with glycerol, fats, soaps and fatty acids. Weiss (1897) also obtained a slight increase with fatty acids (13–15 per cent), but himself remarked that the difference was too slight to be conclusive. Abderhalden and Rona (1904) obtained essentially negative results. The above experiments, therefore, cannot yield us positive evidence.

Embden (1905) first observed, in the livers of phlorhizinized dogs, a production of sugar that was more than could be accounted for by the disappearance of glycogen. Bornstein and Griesbach (1923) noted a fall in the lactic acid content of such livers, and accounted for the excess sugar in this way. Burn and Marks repeated these perfusion experiments using the livers of normal fat-fed cats and dogs, and of one depancreatized cat. Lactic acid (as determined from the content in the perfusing blood), liver glycogen, and protein failed to account for all of the sugar recovered. Since they washed the liver beforehand, they

considered easily diffusible substances like glycol-aldehyde or dioxyacetone as unlikely sources, and hence came to the conclusion that the sugar came from fat. Richardson (1929) has criticized this work on the ground that the anaerobic conditions to which the tissue was subjected in handling probably resulted in the formation of lactic acid, which was reconverted to sugar on perfusion with an oxygenated solution. This might have accounted for the excess sugar.

b. Glycogen formation from fat. Paulesco (1913) excised a lobe of the liver in dogs; then fed glycerol and fatty acids. After 1, 2, and 3 days he killed the animals, and found that only glycerol had caused a deposition of glycogen. The view of Wertheimer (1926) that insulin is a catalyst of fat → glycogen, has already been mentioned. It was based on the fact that in phlorhizinized dogs, through the action of insulin, glycogen appeared in the liver, while fat disappeared. That this can hardly be regarded as necessarily more than a *post hoc* has already been discussed.

Bodey, Lewis and Huber (1927) have observed that a diet of butter oil produced no increase in the liver glycogen of rats.

Rosenfeld (1893, 1912) noted that in a phlorhizinized animal "one can entirely free the liver, already poor in glycogen, by adding fat-feeding to the phlorhizin." Pflüger (1907) and Pflüger and Junkersdorf (1910) also noted that a fat diet tended to free the liver of glycogen.

c. Exclusion of the liver, hepatectomy, and evisceration. Most of the evidence leading to a belief in the conversion of fat to carbohydrate in the liver was derived from experiments in which the liver had been excluded or removed. Porges (1910) and Porges and Salomon (1910) ligated the abdominal aorta, vena cava and liver veins in normal and depancreatized dogs. In both the respiratory quotients were very high after the ligation, and it was concluded that only carbohydrate was oxidized after exclusion of the liver. As a corollary, it followed that the diabetic dog could oxidize sugar and that the liver converted fat to carbohydrate. Rolly (1912) observed an inconstant rise of R.Q. on repeating Porges and Salomon's experiments, but ascribed it to an increased acid production. Murlin, Edelmann and Kramer (1913) proved that the latter was the correct explanation, for they showed that the R.Q., under the conditions, was a function of the blood CO_2 content and that the experiments of Porges and Salomon had no bearing on the oxidation of sugar. Mann and Magath (1923), and Bollmann, Mann and Magath (1925) have studied the carbohydrate metabolism after removal of the liver. There is a marked hypoglycemia, relieved by

intravenous injection of sugar. The hypolgycemia occurs even in the depancreatized animal. The evidence obtained by these investigators indicates that in the hepatectomized animal, muscle glycogen is not easily released to maintain the falling blood sugar. Although this work indicates that the blood sugar is derived from the liver, a conception confirmed by Cori and Cori (1928, 2), it does not imply that the liver store is in turn derived from fat. Nor does it imply that sugar can be oxidized by the depancreatized animal, particularly since these workers found the hypoglycemia to be aggravated by insulin. Minkowski long ago (1887) observed that glycosuria persisted in phlorhizinized birds after extirpation of the liver; and Pavy, Brodie and Siau (1903) observed a similar phenomenon after complete evisceration in dogs. These authors suggested that the sugar might come from the muscle glycogen, but even if this were unlikely, it still is clear that sugar formed from fat by the liver could not have been the source. Mann (1925) observed a rise in the R.Q. immediately following hepatectomy, with a later fall.

Burn and Dale (1924) observed respiratory quotients in the neighborhood of unity after hepatectomy, both in the normal and the depancreatized dog. Markowitz (1928) obtained high R.Q.s after hepatectomy and evisceration (0.87–0.99) in both normal and depancreatized dogs, although in the latter the injection of glucose was followed by a fall in the R.Q. These results are interesting, but their significance is doubtful. Even if the R.Q.s obtained by Burn and Dale and Markowitz were not artifact, the evidence for sugar oxidation in the depancreatized animal is not conclusive, in view of the fact that the observations were made only two days after pancreatectomy. The experiments of Verzár and Fejer (1913) showed that the power to oxidize glucose might persist until the fifth day after pancreatectomy. But with the best technique possible and in the eviscerated animal particularly, the operative disturbance is enormous, as was proved to be the case in the ligation experiments of Porges and Salomon. We are at best dealing with a dying animal, in which a rise or fall in the quotient can hardly be regarded as an index of specific combustion or conversion processes. That this is not mere quibbling is indicated by experiments on other dying animals, for example, those of Moorhouse, Patterson, and Stephenson (1915), and of Hédon (1926), in both of which a rise in the quotient considerably above the diabetic level was observed on the day before death. Certainly there is no reason to suppose that these dying animals suddenly acquired a capacity to burn sugar. One may also revert back to the critical experiments of Murlin, Edelmann

and Kramer, for the probable explanation of these quotients. McMaster and Drury (1927) were unable to find any increase in the R.Q. 8 hours after hepatectomy in rabbits, above that which was observed in the fasting normal animal. In rabbits fed with carbohydrate, the R.Q.s were close to unity both before and after hepatectomy.

The above discussion deals with the evidence regarding the function of the liver and abdominal viscera in the postulated conversion. No evidence has been brought forward, so far as I am aware, implying that other organs, such as muscle, may possess this function to an appreciable extent.

Up to now we have discussed arguments involving the obligatory conversion of fat to carbohydrate. Is there any evidence to indicate that under unusual circumstances it *may* occur?

IV. The Effect of Epinephrin. In so far as the formation of sugar from fat is concerned, the effect of epinephrin may be discussed from three points of view: 1, the effect of repeated injections in the starving animal; 2, the effect after fat feeding; 3, the exclusion of all sources of sugar save fat, quantitatively.

Shortly after his initial paper on epinephrin glycosuria, Blum (1902) observed that after a certain period of starvation (usually 8–9 days) injection of adrenal extract no longer produced a glycosuria, or at most a negligible one. Ritzman (1909) found that in the animal made glycogen-rich by ingestion of sugar, administration of epinephrin produced considerably more glycosuria than in a glycogen-poor animal. Moreover, on continued injection of epinephrin the sugar excretion diminished to zero. Hildebrandt (1920) found that on injecting large doses (1–2 mgm. per kilo) of epinephrin into rabbits, the glycosuria decreased as starvation was prolonged, and Roubitschek (1914) made the same observation. Markowitz (1925) although noting that injections of epinephrin into starved rabbits evokes extensive hyperglycemia, even when the injections are kept up for days, states that hyperglycemia does not occur when the liver is glycogen free. He also notes that repeated injections of epinephrin cause deposition of glycogen in liver, heart and skeletal muscles.

In the diabetic animal a tendency for repeated injections (over more than one day) to result in a diminution of sugar output has also been observed. Ringer (1910) found that while a single injection of epinephrin may result in a rise of the D/N ratio, successive doses may not increase the sugar excretion at all. He concluded that preliminary sweeping out of glycogen occurred, and that subsequent failure to increase

the sugar excretion was due to the depletion of glycogen. A similar experiment was incidentally performed by Sansum and Woodyatt (1915), with a like result. Kramer, Marker, and Murlin (1916) observed the same phenomenon in a depancreatized dog.

Upon feeding olive oil for a few days to animals that, as a result of starvation no longer showed glycosuria on epinephrin injections, Blum (loc. cit.) observed that epinephrin again produced a glycosuria. He conceded the possibility that the glycerol of the fat may have been a source of the sugar, though he believed it improbable. He also did not believe protein could have been the source. As these possibilities were not quantitatively ruled out, however, they can hardly be dismissed. Roubitschek (loc. cit.) made a similar observation, and Eppinger, Falta and Rudinger (1908) observed high D/N ratios in depancreatized dogs after single large injections of epinephrin. The latter experiments were answered by those of Ringer, to which reference has already been made. The authors themselves conceded that glycogen might have been "spared" by fat feeding, and the animals were probably not glycogen free.

As to the experiments of Roubitschek, who used normal animals, there is no reason to believe that an accumulation of glycogen may not have occurred during the week or so of the fat diet, resulting from the deaminization of endogenous protein, which could replenish the depleted glycogen stores. In any case, the amount of fat fed appears to have been less than enough to cover the basal energy requirements (unless the dogs were very small—the weights are not given), under which circumstances it is difficult to see why ingested fat should have been more likely to form glycogen than was the body fat during starvation. Takao (1926) was unable to observe a consistent effect upon sugar excretion in normal rabbits that had been starved, on combining fatty acid feeding with epinephrin injections.

Recently, Chaikoff and Weber have attempted to quantitate the excretion of sugar upon epinephrin injection in relation to its possible origin, using depancreatized dogs. They come to the conclusion that fatty acid cannot be excluded as a source of some of the observed sugar. Macleod (1928) regards these experiments as proving "irrefutably the derivation of sugar from fatty acid, at least in diabetic animals." There is no doubt that if they actually show what they purport to, they constitute proof, not indeed that fatty acids *must* be converted to sugar in the animal body, but certainly that they may be so converted. The experiments are, therefore, important enough to warrant close examina-

tion. Table 19 is modified from Macleod's résumé of the results.

The first point of interest in this paper is that the observations were all made on the fourth day after discontinuance of insulin. The question arises whether the glycogen reserves were as nearly stabilized as the authors assume. That they were not would seem to be indicated by the fact that the D/N ratios appeared in practically no case to have reached a constant level. It might be thought that the answer to the question would lie in the quantitative nature of the data, since the authors would appear to have taken account of the sources of sugar.

TABLE 19

Modified from Macleod's (1928) résumé of Chaikoff and Weber's results

DOG	WEIGHT	EPINEPHRIN IN 12 HOURS	"EXCESS SUGAR"	ACCOUNTED FOR BY LIVER GLYCOGEN, PROTEIN AND GLYCEROL	DIFF.	MUSCLE GLYCOGEN	DIFFERENCE
	kgm.	*mgm.*	*grams*	*grams*†		*grams*	
F	6.8	4	31.3	7.4	+23.9	12.8	+11.1
J	9.0	4	39.8	7.7	+32.1	16.9	+15.2
C	6.3 (1)	1.6	12.6	5.3	+7.3	11.8	−4.5
	6.3 (2)	4	22.8	4.2	+18.6	11.8	+6.8
B	6.2	4	16.3	7.9	+8.4	11.6	−3.2
I	14.0	4	15.2	10.0	+5.2	26.3	−21.1
H	6.8 (1)	8*	10.8	18.8	−8.0	12.8	−20.8
	6.8 (2)	8*	27.0	21.3	+5.7	12.8	−7.1
K	6.7	4	15.0	7.1	+7.9	12.6	−4.7

* 24 hours.

† The table as compiled calculates the sugar obtainable from excess protein on the basis of a D/N of 3.65/1.

However, they do not take into full consideration the muscle glycogen (which they roughly estimate). They state that epinephrin does not raise the blood lactic acid. But against this is the evidence of Cori and Cori (1929, 1), and of Campos, Cannon, Lundin and Walker (1929). On the basis of this assumption they assume that muscle glycogen is not the source of the sugar. The recent work of the Coris has shown that lactic acid derived from muscle glycogen may be transferred to the liver after epinephrin injections and thus, indirectly, become a source of blood sugar. It seems to me that the exclusion of muscle glycogen as a possible source is not fully justified. If it is included, then

in six of the nine experiments, the extra sugar appearing in the urine can be more than accounted for without invoking fatty acids (liver glycogen as a source is quantitatively negligible in these experiments and also might be safely neglected).

But it is also questionable whether the "extra" sugar can be accurately computed. The D/N ratio varies considerably; for example, on the day preceding epinephrin injection it may go below 4/1 or be as high as 21/1; and on the same day, in two twelve hour periods, such variations in the same animal as 10.8/1 and 4.4/1, 19.5/1 and 13.3/1, and 21.1/1 and 9.8/1 are observed. Computation of "extra" sugar from such data is difficult. It is also doubtful whether in all cases the extra nitrogen as a result of epinephrin injection is taken fully into account. The nitrogen of the period following epinephrin is higher than that before epinephrin in three cases; in one it is the same; in four it is unrecorded; and in one it is lower (in this animal, and on the third day of a previous starvation it had been 6.17 grams for twelve hours; on the corresponding experimental day, when epinephrin was administered, it was 0.87 gram).

Finally, the R.Q.s do not seem to be entirely in harmony with the conversion of fat to sugar. Of four experiments cited, in two the quotient was elevated, in one unchanged, and in one lowered. In this last experiment (dog K), where a relatively small amount of glucose is presumably synthesized, the quotient can be harmonized with the theory, but where larger amounts of glucose are not accounted for, this is more difficult. I have calculated that in dog F, on the basis of the basal R.Q. of 0.68, the expected R.Q. after epinephrin, assuming the conversion, should have been 0.62. Actually it was 0.75.

Taking the above considerations into account, it seems fair to question the conclusions drawn from the evidence.

The presence of the liver seems to be necessary in order that the hyperglycemia and glycosuria following epinephrin may appear. Bollman, Mann and Magath (1925) showed that epinephrin did not result in hyperglycemia following liver extirpation. Soskin (1927) observed that epinephrin did not restore the falling blood sugar level in eviscerated dogs, and Collins, Shelling and Byron (1927) noted a similar inhibition of epinephrin effect on ligating the hepatic artery. In a recent series of papers Dr. and Mrs. Cori (1928, 1, 2, 3; 1929, 1, 2) have reported experiments which indicate, in the present connection, that epinephrin hyperglycemia and glycosuria may be due in part to a mobilization of liver glycogen coming from muscle glycogen via lactic acid. That any

part of the sugar that appears following epinephrin injection is synthesized from fat does not seem to be proved.

V. Hibernation. At one time more emphasis was laid upon the respiratory quotients found in hibernating animals than at present seems justified. Very low quotients have at times been obtained, coming close to the theoretical ones for the conversion of fat to sugar. Regnault and Reiset, for example, in 1849 noted an R.Q. of 0.4 in the marmot during deep sleep. Pembrey (1901) observed quotients of 0.53 to 0.55. (References to older literature may be found in Geelmuyden, 1923 (2), p. 174 ff.) The critical analysis of Nagai (1909) first cast serious doubt upon the older results. This observer obtained quotients of between 0.6 and 0.68 during hibernation in marmots, rell-mice and hedgehogs, and regarded the lower quotients as being due to faulty methods. Hari (1909) also questioned the accuracy of the earlier low readings. On the whole, it seems safe to say that there are no authentic quotients in this condition which unequivocally point to the production of sugar from fat. One of the most distinguished of the protagonists of the theory of the conversion offat to carbohydrate feels (Macleod, 1926), that "it is extremely doubtful whether the results of the respiratory exchange of hibernating animals can be accepted as evidence of the conversion of fat into carbohydrate in the animal body." This investigator himself could obtain no quotients of which he could be sure lower than 0.67. Under the highly abnormal conditions due to low body temperature, and the small respiratory exchange, this could not be considered as proof, or as even presumptive, of the conversion.

This once classical type of evidence for the formation of sugar from fatty acids can therefore at present be entirely neglected.

VI. Summary. As was stated at the beginning of this section, the conversion of fat (fatty acids) to carbohydrate must be considered from two points of view: 1. Must the conversion occur? 2. May the conversion occur? The former is based essentially upon three things; 1, that carbohydrate is the sole fuel of exercise; 2, that in diabetes the primary fault is not the failure to oxidize sugar, but an overproduction of sugar from protein and fat; 3, that it is primarily the function of the liver to convert fat to carbohydrate. From the review of the evidence pro and con presented in this section, I believe none of these points is proved. On the contrary, certain illogicalities in the inevitable assumptions would appear to make the obligatory nature of the conversion inherently improbable. The evidence in favor of the possibility that carbohydrate *may* be formed from fat in the animal body under certain conditions seems also to be unsatisfactory.

From the foregoing considerations, the writer believes that no convincing proof exists of the production of carbohydrate from fatty acids in the animal, and that on the contrary, the weight of evidence is at present against it, though he recognizes that the case cannot be considered as closed.

BIBLIOGRAPHY

GENERAL

CREMER, M. Ergebn. Physiol., 1902, i, 802. Physiologie des Glycogens.

GEELMUYDEN, H. C. Ergebn. Physiol., 1923, xxi (1), 274. Die Neubildung von Kohlenhydrat im Tierkörper. I.

GEELMUYDEN, H. C. Ergebn. Physiol., 1923, xxii, 51. Die Neubildung von Kohlenhydrat im Tierkörper. II.

GRAFE, E. Die Pathologische Physiologie des Gesamtstoff- und Kraftwechsel bei der Ernährung des Menschen. München, 1923.

GREVENSTUK, A. AND E. LAQUEUR. Ergebn. Physiol., 1925, xxiii (2), 1. Insulin. [or] Handbuch der Normalen und Pathologischen Physiologie, v, Berlin, 1928.

LANGSTEIN, L. Ergebn. Physiol., 1902, i, 63. Die Bildung von Kohlehydraten aus Eiweiss.

LEATHES, J. B. AND H. S. RAPER. The fats. London, 1925.

LUSK, G. Ergebn. Physiol., 1912, xii, 315. Phlorhizinglukosurie.

LUSK, G. The science of nutrition. 4th ed., Philadelphia, 1928.

MACLEOD, J. J. R. Carbohydrate metabolism and insulin. London, 1926.

MACLEOD, J. J. R. The fuel of life. Princeton, 1928.

MITCHELL, H. H. AND T. S. HAMILTON. The biochemistry of the amino acids. New York, 1929.

PFLÜGER, E. Das Glykogen. Bonn, 1905.

ROSENFELD, G. Ergebn. Physiol., 1902, i (1), 651. Fettbildung.

PROTEIN SYNTHESIS

ABDERHALDEN, E. Zeitschr. physiol. chem., 1912, lxxviii, 1. Futterungsversuche mit voltständig bis zu Aminosäuren abgebautem Eiweiss und mit Ammonsalzen.

ABDERHALDEN, E. Zeitschr. physiol. chem., 1913, lxxxiii, 444. Weitere Versuche über der synthetischen Fähigkeiten des organismus des Hundes.

ABDERHALDEN, E. AND P. HIRSCH. Zeitschr. physiol. chem., 1912, lxxviii, 292. Die Bildung von glykokoll im tierischen Organismus.

ABDERHALDEN, E. AND P. HIRSCH. Zeitschr. physiol. chem., 1912, lxxxii, 1. Fortgesetzte Untersuchungen über die synthetischen Fähigkeiten der tierischen zelle. Versuche über die Verwertung veischiedener Stickstoffquellen im Organismus des Hundes.

ABDERHALDEN, E. AND P. HIRSCH. Zeitschr. physiol. chem., 1912, lxxx, 136. Weitere Beitrag zur Kenntniss der synthetischen Fähigkeiten der zellen von Säugetieren. Fortgesetzte Versuche, den Eiweissbedarf des Hundes durch Ammonsalze und ferner durch einzelne Aminosäurgen ganz oder teilweise zu decken.

ABDERHALDEN, E. AND P. HIRSCH. Zeitschr. physiol. chem., 1912, lxxxi, 323. Fütterungsversuche mit Gelatine, Ammonsalzen, vollständig abgebautem Fleisch und einem aus allen bekannten Aminosäuren bestehenden Gemisch ausgeführt an jungen Hunden.

ABDERHALDEN, E. AND P. HIRSCH. Zeitschr. physiol. chem., 1913, lxxxiv, 189. Weiterer Beitrag zur Kenntnis due synthetischen Fähigkeiten der tierischen zelle. Die Wirkung des Salpeters auf den Stickstoffwechsel.

ABDERHALDEN, E. AND A. E. LAMPÉ. Zeitschr. physiol. chem., 1912, lxxxii, 21. Weiterer Beitrag zur Kenntnis der synthetischen Fahigkeiten der tierischen zelle. Versuche über die Verwertung verschiedenartiger Stickstoffquellen im Organismus des Hundes.

ABDERHALDEN, E. AND A. E. LAMPÉ. Zeitschr. physiol. chem., 1913, lxxxiii, 409. Weiterer Beitrag zur Kenntnis der Wirkung von Ammonsalzen, Glukosamin und Gelatine auf die Stickstoffbilanz.

ABDERHALDEN, E. AND A. E. LAMPE. Zeitschr. physiol. chem., 1913, lxxxiv, 218. Über den Einfluss von per os verabreichtem Harnstoff auf den Stickstoffwechsel beim Schweine.

ARMSBY, H. P. Principles of animal nutrition. New York, 1908.

ARMSBY, H. P. U. S. Dept. Agric., Bur. Anim. Ind., Bull. 139, 1911.

BORSOOK, H. AND H. WASTENEYS. Biochem. Journ., 1925, xix, 1128. The interaction of free amino-nitrogen and glucose.

CATHCART, E. P. Journ. Physiol., 1909, xxix, 311. The influence of carbohydrates and fats on protein metabolism.

CATHCART, G. D. Biochem. Journ., 1916, x, 197. Nitrogen distribution in the tissues and some of the factors which influence it.

DAKIN, H. D. AND H. W. DUDLEY. Journ. Biol. Chem., 1913, xiv, 555. A contribution to a theory concerning the intermediary metabolism of carbohydrates and proteins. The mutual interconversion of α-amino-acids, α-hydroxy-acids and α-ketonic aldehydes.

DAKIN, H. D. AND H. W. DUDLEY. Journ. Biol. Chem., 1913, xv, 127. The interconversion of α-amino-acids, α-hydroxy-acids and α-ketonic aldehydes.

EMBDEN, G. AND E. SCHMITZ. Biochem. Zeitschr., 1910, xxix, 423. Über synthetische Bildung von Aminosäuren in der Leber.

EMBDEN, G. AND E. SCHMITZ. Biochem. Zeitschr., 1912, xxxviii, 393. Über synthetische Bildung von Aminosäuren in der Leber.

EMBDEN, G. AND F. KRAUS. Biochem. Zeitschr., 1912, xlv, 1. Über Milchsäurebildung in der Künstlich durchstromten Leber.

FELLNER, H. Biochem. Zeitschr., 1912, xxxviii, 414. Über synthetische Bildung von Aminosäuren in der Leber.

FRIEDLANDER, K. Landvert. Versuchsstat., 1907, lxvii, 283. Zur Frage des Eiweisszusatzes durch amide.

GABRIEL, S. Zeitschr. f. Biol., 1892, xxix, 115. Zur Frage nach der Bedeutung des Asparagins als Nährungsstoff.

GESSLER, H. Zeitschr. f. physiol. Chem., 1920, cix, 280. Zur Frage des Wesens der Stickstoffretention bei Fütterung mit Ammoniaksalzen.

GRAFE, E. Zeitschr. f. physiol. Chem., 1912, lxxviii, 485. Weitere Mitteilungen über die Eiweiss sparende Wirkung verfütterter Ammoniaksalze.

GRAFE, E. Zeitschr. f. physiol. Chem., 1912, lxxxii, 347. Weitre Mitteilungen über die Stickstoffrententionen bei Fütterung von Ammoniaksalzen.

GRAFE, E. Zeitschr. f. physiol. Chem., 1913, lxxxiv, 69. Über Stickstoffansatz bei Fütterung kleiner Eiweissgaben und gröszerer Mengen von Ammoniaksalzen und Harnstoff.

GRAFE, E. Zeitschr. f. physiol. Chem., 1913, lxxxvi, 347. Zur Frage der Stickstoffretentionen bei Fütterung von Harnstoff.

GRAFE, E. Zeitschr. f. physiol. Chem., 1913, lxxxviii, 389. Beitrage zur Kenntnis der Art der Stickstoffretentionen bei Fütterung von Ammoniaksalzen und Harnstoff.

GRAFE, E. Zeitschr. f. physiol. Chem., 1914, xc, 75. Zur Frage der Stickstoffretentionen bei Fütterung von Ammoniumchlorid.

GRAFE, E. AND V. SCHLÄPFER. Zeitschr. f. physiol. Chem., 1912, lxxvii, 1. Über Stickstoffretentionen und Stickstoffgleichgewicht bei Fütterung von Ammoniaksalzen.

GRAFE, E. AND K. TURBAN. Zeitschr. f. physiol. Chem., 1913, lxxxiii, 25. Über Stickstoffretentionen bei Fütterung von Harnstoff.

GRAFE, E. AND H. WINTZ. Zeitschr. f. physiol. Chem., 1913, lxxxvi, 283. Über die Beeinflüssung des Stickstoff-Stoffwechsel durch Fütterung von Natriumnitrat.

HENRIQUES, V. AND A. C. ANDERSEN. Zeitschr. f. physiol. Chem., 1914, xcii, 21. Über Stickstoffretentionen bei zufuehr von Ammoniaksalzen oder Harnstoff. Curch Versuche mit permanent-intravenöser Injektion untersucht.

HENRIQUES, V. AND C. HANSEN. Zeitschr. f. physiol. Chem., 1907, liv, 169. Über die Bedeutung der sogenannten "Pflanzenamide" fur den Stickstoffumsatz im tierischen Organismus.

JANNEY, N. W. N. Y. Med. Journ., 1918, cvii, 824. The relation of carbohydrates to protein synthesis.

JANNEY, N. W. N. Y. Med. Journ., 1918, cvii, 879. The relation of carbohydrates to protein synthesis.

JUST, J. Landwirt. Versuchsstat., 1908, lxix, 393. Vergleichende Untersuchüngen über die Wirkung des Eiweisses und Einiger nichteuweissartiger Stickstoff verbindunger auf den Fleischansatz beim Wieder Kauer.

KAYSER, H., DUBOIS. Arch. f. (anat. u) Physiol., 1893, 371. Über die eiweissersparende Kraft des Fettes, verglichen mit derjenigen des Kohlehydrats.

KELLNER, O. Zeitschr. f. Biol., 1900, xxxix, 313. Untersuchungen über den Einfluss des Asparagins und Ammoniaks auf den Eiweissumsatz der Wiederkäuer.

KELLNER, O., P. EISENKOBBE, R. FLEBBE AND R. NEUMANN. Landwirt. Versuchsstat., 1910, lxxii, 437. Untersuchungen über den Einfluss einiger might-eiweissartiger Stickstoffnerbindungen auf den Eiweissumsatz beim Wiederkäuer.

KNOOP, F. Zeitschr. f. physiol. Chem., 1910, lxvii, 489. Über den physiologischer abbau der Säuren und die synthese einer Aminosäure eim Tierkörper.

KNOOP, F. Zeitschr. f. physiol. Chem., 1914, lxxxix, 151. Über Aminösaureabbau und Glykokollbildung.

KNOOP, R. Münch. Med. Wochenschr., 1926, lxxiii, 2151. Ueber die Stickstoffbindung bei der Eiweisssynthese in der Natur und das Interesse der Biologie an den physiologischen Oxydationsreaktionen und ihrer Umkehrbarkeit.

KNOOP, F. AND E. KERTESS. Zeitschr. f. Physiol. Chem., 1911, lxxi, 252. Das Verhalten von α-aminosäuren und α-ketonsäuren im Tierkörper.

KNOOP, F. AND J. G. BLANÇO. Zeitschr. f. physiol. Chem., 1925, cxlvi, 267. Über die Acetylierung von Aminosäuren im Tierkörper.

KNOOP, F. AND H. OSTERLIN. Zeitschr. f. physiol. Chem., 1925, cxlviii, 294. Über die natürliche Synthese der Aminosäuren und ihre experimentelle Reproduktion.

KOCHER, R. A. Journ. Biol. Chem., 1916, xxv, 571. The mechanism of the sparing action of carbohydrates on protein metabolism.

KONDO, K. Biochem. Zeitschr., 1912, xxxviii, 407. Über synthetische aminosäurebildung in der Leber.

KOSTYTSCHEW, S. AND W. BRILLIANT. Zeitschr. f. physiol. Chem., 1923, cxxvii, 224. Die Verwandlungen der Aminosäuren in Gegenwart von Zucker.

LANDERGREN, E. Skand. Arch. f. Physiol., 1903, xiv, 112. Untersuchungen über die Eiweissumsetzung des Menschen.

LEHMANN, C. Pflüger's Arch., 1906, cxii, 339. Beiträge zur Kenntniss der Wirkung des Asparagins auf den Stickstoffumsatz im Thierkörper.

MAUTHNER, J. Zeitschr. f. Biol., 1891, xxviii, 507. Ueber den Einfluss des Asparagins auf den Umsatz des Eiweisses beim Fleischfresser.

MÜLLER, M. Pflüger's Arch., 1907, cxvii, 497. Weitere Untersuchungen über die Wirkung des Asparagins auf den Stickstoffumsatz und ansatz des Tierkörper.

MUNK, I. Arch. f. path. Anat., 1883, xciv, 436. Der Einfluss des Asparagin auf den Eiweissumsatz und die Bedeutung Desselben als Nährstoff.

MÜRLIN, J. R. Amer. Journ. Physiol., 1907, xx, 234. The nutritive value of gelatin. II. Significance of glycocoll and carbohydrate in sparing the body's proteid.

NEUBERG, C. AND L. LANGSTEIN. Arch. f. Physiol., 1903, (Suppl.) 514. Ein Fall von Desamidirung im Thierkörper; zugleich ein Beiträg zur Frage nach der Herkunft des Glykogens.

NEUBERG, C. AND M. KOBEL. Biochem. Zeitschr., 1926, clxxiv, 464. Weiteres die über Reaktion einzelner Zuckerarten mit verschiedenen animosäuren nebst Bemerkungen.

PATON, D. N. Journ. Physiol., 1905, xxxiii, 1. On Folin's theory of proteid metabolism.

PESCHEK, E. Biochem. Zeitschr., 1912, xlv, 244. Über Einwirkung von Ammoniaksalzen und essigsauren Salzen auf den Stickstoffwechsel des Fleischfressers.

POLITIS, G. Zeitschr. f. Biol., 1891, xxviii, 492. Ueber die Bedeutung des Asparagins als Nahrungsstof.

RINGER, A. I. Journ. Biol. Chem., 1912, xii, 431. Protein metabolism in experimental diabetes.

RINGER, A. I. AND E. M. FRANKEL. Journ. Biol. Chem., 1914, xvi, 563. The effects of acetaldehyde and propylaldehyde on the sugar formation and acidosis in the diabetic organism.

RUBNER, M. Arch. f. Hygiene, 1908, lxvi, 1. Protein metabolism in experimental diabetes.

SALKOWSKI, E. Zeitschr. physiol. Chem., 1920, cix, 276. Zur Kenntnis der Eiweissbildung aus Harnstoff bei Wiederkäuren.

SCHEUNERT, A., W. KLEIN AND M. STEUBER. Biochem. Zeitschr., 1922, cxxxiii, 137. Über die Verwertbarkeit des Harnstoffs als Eiweiss quelle fur Wiederkäuer zugleich ein Beitr g zur Frage der exkretorischen Funktionen der Haut.

TAYLOR, A. E. AND A. S. RINGER. Journ. Biol. Chem., 1913, xiv, 407. The utilization of ammonia in the protein metabolism.

TERROINE, E. F. AND O. MAHLER-MENDLER. Arch. Intern. Physiol., 1927, xxviii, 101. Le metabolisme de l'azote pendant la croissance.

UNDERHILL, F. P. Journ. Biol. Chem., 1913, xv, 337. A note on the elimination of ingested ammonium salts during a period of prolonged inanition.

UNDERHILL, F. P. AND S. GOLDSCHMIDT. Journ. Biol. Chem., 1913, xv, 341. The utilization of ammonium salts with a non-nitrogenous diet.

VOIT, C. Zeitschr. f. Biol., 1892, xxix, 125. Bemerküng zu der Mittheilung von Dr. S. Gabriel.

VÖLTZ, W. Pflüger's Arch., 1905, cvii, 415. Über den Einfluss des Lezithens auf den Eiweissumsatz ohne gleichzeitige Asparaginzufuhr und bei gegenwart dieses Amids.

VÖLTZ, W. Pflüger's Arch., 1905, cvii, 360. Über den Einfluss verschiedener Eiweisskörper und einiger Derivate derselben auf den Stickstoffumsatz; mit besonderer Berucksichtigung des Asparagins.

VÖLTZ, W. Pflüger's Arch., 1906, cxii, 413. Über das Verhalten einiger Amidsubstanzen allein und im Gemisch im Stoffwechsel der Karnivoren.

WEISKE, H. Zeitschr. f. Biol., 1894, xxx, 254. Ueber die Bedeutung des Asparagins fur die Ernährung der Herbivoren.

WEISKE, H., M. SCHRODT AND ST. DANGEL. Zeitschr. f. Biol., 1879, xv, 261. Ueber die Bedeutung des Asparagins fur die thierische Ernährung.

WEISKE, H., G. KENNEPOHL AND B. SCHULZE. Zeitschr. f. Biol., 1881, xvii, 415. Ueber die Bedeutung des Asparagins fur die thierische Ernährung.

ZUNTZ, N. Pflüger's Arch., 1891, xlix, 477. Bemerkungen über die Verdauung und den Nährwerth der Cellulose.

PROTEIN TO FAT

ABDERHALDEN, E. AND C. BRAHM. Zeitschr. f. Physiol. chem., 1910, lxv, 330. Ist das am Aufbau der Körperzellen beteiligte Fett in seiner Zusammensetzung von der Art des aufgenommenen Nahrungsfettes abhängig?

ATHANASIU, J. Pflüger's Arch., 1899, lxxiv, 511. Die Erzeugung von Fett im Thierischen Körper unter dem Einfluss von Phosphor.

ATKINSON, H. V., D. RAPPORT AND G. LUSK. Journ. Biol. Chem., 1922, liii, 155. The production of fat from protein.

BEEBE, S. P. AND B. H. BUXTON. Amer. Journ. Physiol., 1905, xii, 466. The production of fat from proteid by the bacillus pyocyaneus.

BERG, W. AND V. FALK. Zeitschr. f. Mikr. anat. Forsch., 1924, i, 297. Mikroskopische Untersuchungen über den zusammenhang von Eiweissabbau und Fettgehalt in den quergestreiften Muskelfasem des Frosches im Winter.

CESARIS-DEMEL, A. Arch. Ital. de Biol., 1909, li, 197. L'origine endogene de la graisse démontrée sur le coeur isolé de mammifère.

CREMER, M. Münch. Med. Wochenschr., 1897, xliv, 811. Ueber Fettbildung aus Eiweiss bei der Katze.

CREMER, M. Zeitschr. f. Biol., 1899, xxxviii, 309. Fettbildung aus Eiweiss bei der Katze.

DAKIN, H. D. Journ. Biol. Chem., 1923, lvi, 43. Experiments on the catabolism of caproic acid and its derivatives.

GORTNER, R. A. Journ. Amer. Chem. Soc., 1914, xxxvi, 1556. Comparative analyses of the eggs and of the newly-hatched larvae of the giant salamander, cryptobranchus allegheniensis.

GROSS, O. AND F. VORPAHL. Arch. f. exp. Path. u Pharm., 1914, lxxvi, 336. Beitrag zur Lehre von der Verfettung parenchymatöser Organe.

GROSS, O. AND F. VORPAHL. Arch. f. exp. Path. u Pharm., 1914, lxxvii, 317. Beitrag zur Lehre von der Verfettung parenchymatäser Organe.

HILDESHEIM, O. AND J. B. LEATHES. Journ. Physiol., 1904, xxxi, 1. On the synthesis of higher fatty acids in the liver.

HOFMANN, F. Zeitschr. f. Biol., 1872, viii, 153. Der Uebergang von Nahrungsfett in die Zellen des Thierkörpers.

JACOBSTHAL, H. Pflüger's Arch., 1893, liv, 484. Versuche über die Fettbildung bei der Reifung des Käses.

KAUFMANN, M. M. Arch. de Physiol., 1896, xxviii, 329. Transformations chimiques intra-organiques.

KAUFMANN, M. M. Arch. de Physiol., 1896, xxviii, 757. Transformation des matieres hydrocarbonées des aliments en graisse dans l'organisme animal.

KAUFMANN, M. M. Arch. de Physiol., 1896, xxviii, 767. Transformation des albuminöides en graisse.

KEMMERICH. Zentralbl. f. d. med. Wiss., 1866, iv, 465. Untersuchungen über die Bildung der Milchfette.

KONDO, K. Biochem. Zeitschr., 1914, lix, 113. Ein experimenteller Beitrag zur Frage der Fettbildung aus Eiweiss bei der Reifung des Käses.

KRAUS, F. AND A. SOMMER. Beitr. z. chem. Physiol. u Path., 1902, ii, 86. Über Fettwanderung bei Phosphorintoxikation.

KUMAGAWA, M. AND G. KANEDA. Maly's Jahresber., 1894, xxiv, 41. Zur Frage der Fettbildung aus Eiweiss im Thierkörper.

LAFON, M. G. Compt. Rend., 1913, clvi, 952. Sur la formation de la graisse aux dépens des matières albuminöides dans l'organisme animal.

LEBEDEFF, A. Pflüger's Arch., 1883, xxxi, 11. Woraus bildet sich das Fett in Fällen des akuten Fettbildung?

LEICK AND WINCKLER. Arch. f. exp. Path. u Pharm., 1902, xlviii, 163. Die Herkunft des Fettes bei Fettmetamorphose des Herzfleisches.

LEO, H. Zeitschr. f. physiol. Chem., 1885, ix, 469. Fettbildung und Fettransport bei Phosphorintoxication.

LEO, H. Biochem. Zeitschr., 1913, xlviii, 297. Über das Wesen der Organverfettungen nach Phosphorvergiftung.

LEO, H. AND W. TRUSCHENNIKOFF. Biochem. Zeitschr., 1913, xlviii, 302. Untersuchungen über Fettbildung unter dem Einfluss des Phosphors.

LEO, H. AND C. BACHEM. Biochem. Zeitschr., 1913, xlviii, 313. Weitere Untersuchungen über Fettbildung in der überlebenden Leber.

LIEBIG, J. Ann. d. Chem. u Pharm., 1843, xlviii, 126. Die Fettbildung im Thierkörper.

LINDEMANN, W. Zeitschr. f. Biol., 1899, xxxviii, 405. Ueber das Fett des normalen und des fettig entarteten Herzmuskels.

MAIGNON, M. F. Compt. Rend. Acad. Sci., 1918, clxvi, 919, 1008. Recherches sur la toxicité de l'albumine d'oeuf. Influence des saisons sur la sensibilité de l'organisme à l'intoxication azotée.

MAIGNON, M. F. Compt. Rend. Acad. Sci., 1918, clxvii, 91. Influence de l'espèce animale sur la toxicité et le mode d'utilisation des proteines alimentaires.

MAIGNON, M. F. Compt. Rend. Acad. Sci., 1919, clxviii, 626. Etude du mecanisme de l'action des graisses dans l'utilisation et l'assimilation des albuminöides.

MAIGNON, F. AND L. JUNG. Compt. Rend. Soc. Biol., 1924, xci, 1390. Relation entre l'influence des saisons sur la sensibilité der l'organisme a l'intoxication azotée, etc.

MACLEAN, I. S. Biochem. Journ., 1922, xvi, 370. The conditions influencing the formation of fat by the yeast cell.

MCCLENDON, J. R. Journ. Biol. Chem., 1915, xxi, 269. On the formation of fats from proteins in the eggs of fish and amphibians.

MUNK, I. Virchow's Arch., 1884, xcv, 407. Zur Lehre von der Resorption, Bildung und Ablagerung der Fette im Thierkörper.

NÄGELI, C. AND O. LOEW. Ann d. Chem. u Pharm., 1878, cxciii, 322. Über die chemische zusammensetzung der Hefe.

NEUBERG, C. Biochem. Zeitschr., 1911, xxxvii, 501. Über die Herkunft der optisch-aktiven Valeriansäure bei der Eiweissfäulnis.

NEUBERG, C. AND E. ROSENBERG. Biochem. Zeitschr., 1907, vii, 178. Über die bei der Eiweissfäulnis auftretenden Fettsäuren sowie über die optisch-aktive Valeriansäure und Capronsäure.

NISHIKATA, T. Journ. Biochem. (Japan), 1922, i, 261. Ein experimenteller beitrag zum studium der Fettbildung aus eiweiss beim wachstum der fliegenmaden.

OHTA, K. Biochem. Zeitschr., 1910, xxix, 1. Über das Verhalten des Organfettes bei der Autolyse und antiseptischem Aufbewahren.

OSBORNE, T. B. AND L. B. MENDEL. Journ. Biol. Chem., 1924, lix, 13. Nutrition and growth on diets highly deficient or entirely lacking in preformed carbohydrates.

PETTENKOFER, M. AND C. VOIT. Ann. d. Chem. u Pharm., 1862, Suppl. 2, 361. Ueber die Producte der Respiration des Hundes bei Fleischnahrung und über die Gleichung der Einnahmen und ausgaben des Körpers dabei.

PETTENKOFER, M. AND C. VOIT. Zeitschr. f. Biol., 1871, vii, 433. Ueber die Zersetzungsvorgänge im Thierkörper bei Fütterung mit Fleisch.

PFLÜGER, E. Pflüger's Arch., 1892, li, 229. Ueber die Entstehung von Fett aus Eiweiss im Körper der Thiere.

PFLÜGER, E. Pflüger's Arch., 1892, lii, 239. Die Einährung mit Kohlehydraten und Fleisch oder auch mit Kohlehydraten allein.

PFLÜGER, E. Pflüger's Arch., 1897, lxviii, 176. Neue Versuche zur Begründung der Lehre von der Entstehung des Fettes aus Eiweiss.

POLIMANTI, O. Pflüger's Arch., 1898, lxx, 349. Ueber die Bildung von Fett im organismus nach Phosphorvergiftung.

RADZIEJEWSKI, S. Arch. f. path. Anat., 1872, lvi, 211. Zusatz zu den experimentellen Beiträgen zur Fettresorption.

RAY, W. E., T. S. MCDERMOTT AND G. LUSK. Amer. Journ. Physiol., 1899, iii, 139. On metabolism during a combination of phosphorus poisoning and phlorhizin diabetes.

ROSENFELD, G. Verhndl. d. Kongr. f. inn. Med., 1893, xii, 359. Ueber Phloridzinwirkungen.

ROSENFELD, G. Zeitschr. f. klin. Med., 1895, xxviii, 256. Die Fettleber beim Phloridzindiabetes.

ROSENFELD, G. Verhandl. d. Kongr. f. inn. Med., 1895, xiii, 414. Ueber Fettwanderung.

ROSENFELD, G. Verhndl. d. Kongr. f. inn. Med., 1897, xv, 427. Giebt es eine fettige Degeneration?

ROSENFELD, G. Verhndl. d. Kongr. f. inn. Med., 1899, xvii, 503. Die Herkunft des Fettes.

ROSENFELD, G. Berl. klin. Wochenschr., 1910, xlvii, 1268. Eiweisskörper und Leberverfettung.

RUBNER, M. Zeitschr. f. Biol., 1885, xxi, 250. Calorimetrische Untersuchungen. I.

SAXL, P. Biochem. Zeitschr., 1908, xii, 343. Über Fett und Esterspaltung in den Geweben.

SHIBATA, N. Biochem. Zeitschr., 1911, xxxvii, 345. Ein experimenteller Beitrag zur Kenntnis der Fettwanderung bei der Phosphorvergiftung mit Berucksichtigung der Herkunft des Fettes im Tierorganismus.

SIEGERT, F. Beitr. z. chem. Physiol. u. Path., 1902, i, 114. Das Verhalten des Fettes bei der Autolyse der Leber.

SSUBBOTIN, V. Arch. f. path. Anat., 1866, xxxvi, 561. Ueber den Einfluss der Nahrung auf die quantitative Zusammensetzung der milch.

SSUBBOTIN, V. Zeitschr. f. Biol., 1870, vi, 73. Beiträge zur Physiologie des Fettgewebes.

TANGL, F. AND K. FARKAS. Pflüger's Arch., 1904, civ, 624. Beiträge zur Energetik der Ontogenese.

TERROINE, E. F. AND P. BELIN. Arch. Intern. Physiol., 1926, xxvi, 280.

UNDERHILL, F. P. AND B. M. HENDRIX. Journ. Biol. Chem., 1915, xxii, 471. Does fat formation occur in the perfused kidney?

VOIT, C. Zeitschr. f. Biol., 1869, v. 79. Ueber die Fettbildung im Thierkörper.

VOIT, C. Zeitschr. f. Biol., 1870, vi, 305. Ueber die Entwicklung der Lehre von der Quelle der Muskelkraft.

VOIT, C. Hermann's Handbch d. Physiol., Leipzig, 1881, vi (1). Physiologie des allgemeinen Stoffwechsels.

VOIT, E. Münch. Med. Wochenschr., 1892, xxxix, 460. Ueber die Fettbildung aus Eiweiss.

WALKER AND E. W. AINLEY. Journ. Biol., 1922, lvi (Proceedings). The formation of fat from protein.

WEINLAND, E. Zeitschr. f. Biol., 1908, li, 187. Über die Bildung von Fett aus eiweissartiger Substanz im Brei der Calliphora.

WEISS, R. AND D. RAPPORT. Journ. Biol. Chem., 1924, lx, 513. The interrelations between certain amino acids and proteins with reference to their specific dynamic action.

WELLS, H. G. Zeitschr. f. Physiol. Chem., 1905, lv, 412. Versuche über den Transport von jodiertem Fett bei Phosphorvergiftung.

WILLIAMS, H. B., J. A. RICHE AND G. LUSK. Journ. Biol. Chem., 1912, xii, 349. Metabolism of the dog following the ingestion of meat in large quantity.

WOLTKE, W. Maly's Jahresber., 1901, xxxi, 77. Über die Veränderungen des Fettes infolge der Phosphorvergiftung.

PROTEIN TO CARBOHYDRATE

ADAMS, E. T. Journ. Biol. Chem., 1926, lxvii, p. xxi. Specific dynamic action from the standpoint of the second and third laws of thermodynamics.

ARTEAGA, J. F. Amer. Journ. Physiol., 1901, vi, 173. Phlorhizin diabetes in cats.

ATKINSON, H. V., D. RAPPORT AND G. LUSK. Journ. Biol. Chem., 1922, liii, 155. The production of fat from protein.

AUBEL, E. Bull. Soc. hyg. aliment., 1924, xii, 257, 416.

AUBEL, E. Ann. de Physiol. et de Physiochem. Biol., 1927, iii, 121. Sur l'action dynamique specifique.

BENEDICT, S. R. AND R. C. LEWIS. Proc. Soc. Exp. Biol. and Med., 1914, xi, 134. The influence of induced diabetes on malignant tumors.

BENDIX, E. Zeitschr. f. Physiol. Chem., 1901, xxxii, 479. Über physiologische Zuckerbildung nach Eiweissdarreicbung.

BENDIX, E. Zeitschr. f. Physiol. Chem., 1902, xxxiv, 544. Bemerkungen zu: "Die Entstehung von glycogen aus Eiweiss," von Bernhard Schöndorff.

BERNARD, C. Lecons sur le Diabete. Paris, 1877.

CAMPBELL, W. R. AND J. MARKOWITZ. Amer. Journ. Physiol., 1926, lxxx, 561. On the metabolism of dihydroxyacetone in pancreatic diabetes.

CHAIKOFF, I. L. Journ. Biol. Chem., 1927, lxxiv, 203. Observation on the ketone body excretion, the dextrose to nitrogen ratios and the glycogen content of liver and muscles of fasted depancreatized dogs.

CHAIKOFF, I. L., J. J. R. MACLEOD, J. MARKOWITZ AND W. W. SIMPSON. Amer. Journ. Physiol., 1925, lxxiv, 36. Observations on depancreatized dogs before and after the withdrawl of insulin.

CHAMBERS, H. W. AND G. LUSK. Journ. Biol. Chem., 1930, lxxv, 611. Specific dynamic action in the normal and phlorhizinized dog.

COHN, E. J., J. T. HENDRY AND A. M. PRENTISS. Journ. Biol. Chem., 1925, lxiii, 721. The molecular weights of the proteins.

CREMER, M. Zeitschr. f. Biol., 1899, xxxviii, 309. Fettbildung aus Eiweiss bei der Katze.

DAKIN, H. D. Journ. Biol. Chem., 1912, xiii, 513. The fate of proline in the animal body.

DAKIN, H. D. Journ. Biol. Chem., 1913, xiv, 321. Studies on the intermediary metabolism of amino-acids.

DAKIN, H. D. Biochem. Journ., 1919, xiii, 398. On amino-acids. II. Hydroxyglutamic acid.

DAKIN, H. D. AND H. W. DUDLEY. Journ. Biol. Chem., 1913, xiv, 555. A contribution to a theory concerning the intermediary metabolism of carbohydrates and proteins. The mutual interconversion of α-amino-acids, α-hydroxy-acids and α-ketonic aldehydes.

DAKIN, H. D. AND H. W. DUDLEY. Journ. Biol. Chem., 1913, xv, 127. The interconversion of α-amino-acids, α-hydroxy-acids and α-ketonic aldehydes.

DAKIN, H. D. AND H. W. DUDLEY. Journ. Biol. Chem., 1914, xviii, 29. The formation of amino and hydroxy-acids from glyoxals in the animal organism.

DEUEL, H. J., H. E. WILSON AND A. T. MILHORAT. Journ. Biol. Chem., 1927, lxxiv, 265. On the mechanism of phorhizin diabetes.

DUBOIS, E. F. Basal metabolism in health and disease. Philadelphia, 1927.

EMBDEN, G. AND H. SALOMON. Beitr. z. chem. Physiol. u Path., 1904, v, 507. Über alaninfütterungsversuche am pankreaslosen Hunde.

EMBDEN, G. AND H. SALOMON. Beitr. z. chem. Physiol. u Path., 1905, vi, 63. Fütterungsversuche am pankreaslosen Hunde.

V. FALKENHAUSEN, M. Arch. f. Exp. Path. u Pharm., 1925, cix, 249. Untersuchungen über den Eiweiszstoffwechsel beim experimentellen Pankreasdiabetes.

GEYELIN, H. R. AND E. F. DUBOIS. Journ. Amer. Med. Assoc., 1916, lxvi, 1532. A case of diabetes of maximum severity with marked improvement.

GEELMUYDEN, H. C. Ergebn. Physiol., 1925, xxiv, 1. Über die spezifisch-dynamische Wirküng der Nahrungsstoffe und ihre Beziehungen zum Grundumsatz beim diabetes mellitus.

GLAESSNER, K. AND E. P. PICK. Beitr. z. chem. Physiol. u Path., 1907, x, 473. Über Phlorizindiabetes.

GREENWALD, I. Journ. Biol. Chem., 1913, xvi, 375. The formation of glucose from propionic acid in diabetes mellitus.

HALSEY, J. T. Sitzungsber. d. Gesellsch. z. Beförderung der gesamt Wissensch. zu Marburg, 1899, 102. (Quoted by G. LUSK, Ergebn. Physiol., 1912, xii, 315.)

HALSEY, J. T. Amer. Journ. Physiol., 1904, x, 229. Concerning the formation of sugar from leucin.

HANISCH, L. AND P. JUNKERSDORF. Pflüger's Arch., 1927, ccxvii, 264. Das Stoffwechselgeschehen im Phlorihizin-Diabetes bei einseitiger Ernährung mit Eiweiss.

JANNEY, N. W. Journ. Biol. Chem., 1915, xx, 321. The metabolic relationship of the proteins to glucose.

JANNEY, N. W. AND F. A. CSONKA. Journ. Biol. Chem., 1915, xxii, 203. The metabolic relationship of the proteins to glucose. II. Glucose formation from body proteins.

KNOPF, L. Arch. f. Exp. Path. u Pharm., 1903, xlix, 123. Beiträge zur Kenntniss des Phlorhizindiabetes.

KRAUS, F. Berl. Klin. Wochenschr., 1904, xli, 4. Ueber die Frage der Zuckerbildung aus den Eiweiss im diabetschen organismus.

KÜLZ, E. Arch. f. Exp. Path. u Pharm., 1876, vi, 140. Kann in der schweren Form des Diabetes die Zuckerausfuhr durch vermehrte zufuhr von albuminaten gestigertwerden.

KÜLZ, E. Pflüger's Arch., 1881, xxiv, 70. Ueber eine Versuchsofrom Bernard's, welche die Entstehung des glycogens aus Eiweiss beweisen soll.

LABBÉ, M. AND F. NEPVEUX. Compt. Rend. Soc. Biol., 1922, lxxxvii, 346. Les Réactions d'hyperglycémie provoquées par les ingestions d'albumines.

LOEWI, O. Arch. f. Exp. Path. u Pharm., 1902, xlvii, 56. Ueber den Einfluss des Campher auf die Grösse der Zuckerausscheidung im Phlorhizindiabetes.

LOEWI, O. Arch. f. Exp. Path. u Pharm., 1902, xlvii, 68. Zur Frage nach der Bildung von Zucker aus Fett.

LOEWY, A. Oppenheimer's Handbuch d. Biochem., 1911, iv (1), 279. Die respiratorische und der Gesamtumsatz.

LUSK, G. Zeitschr. f. Biol., 1901, xlii, 31. Über Phlorhizin Diabetes.

LUSK, G. Amer. Journ. Physiol., 1908, xxii, 174. The production of sugar from glutamic acid ingested in phlorhizin glycosuria.

LUSK, G. Journ. Amer. Chem. Soc., 1910, xxxii, 671. The fat of the amino acids in the organism.

LUSK, G. Arch. Int. Med., 1915, xv, 939. On the diabetic respiratory quotient.

LÜTHJE, H. Zeitschr. f. Klin. Med., 1900, xxxix, 397. Stoffwechselversuch an einem Diabetker, mit specieller Berücksichtigung der Frage der zuckerbildung aus Eiweiss und Fett.

LÜTHJE, H. Deutsch. Arch. Klin. Med., 1904, lxxix, 498. Die Zuckerbildung aus Eiweiss.

LÜTHJE, H. Pflüger's Arch., 1905, cvi, 160. Zur Frage der Zuckerbildung aus Eiweiss.

MAGNUS-LEVY, A. Zeitschr. f. Klin. Med., 1905, lvi, 83. Respirationsversuche an diabetischen Menschen.

MANDEL, A. R. AND G. LUSK. Deutsch. Arch. f. Klin. Med., 1904, lxxxi, 472. Stoffwechselbeobachtungen an einem Falle von Diabetes mellitus, mit besonderer Berücksichtigung der Prognose.

MANN, F. C. AND T. B. MAGATH. Arch. Int. Med., 1922, xxx, 73. Studies on the physiology of the liver.

MANN, F. C. AND T. B. MAGATH. Arch. Int. Med., 1922, xxx, 171. Studies on the physiology of the liver.

MANN, F. C. AND T. B. MAGATH. Arch. Int. Med., 1923, xxxi, 797. Studies on the physiology of the liver.

MACLEOD, J. J. R. AND J. MARKOWITZ. Trans. Assoc. Amer. Phys., 1926, xli, 147. Further researches on depancreatized animals treated with insulin.

MERING, I. Verhand. d. Kongr. f. inn. Med , 1886, v, 185. Ueber experimentellen Diabetes.

MERING, I. Verhand. d. Kongr. f. inn. Med., 1887, vi, 349. Ueber Diabetes mellitus.

MERING, I. Zeitschr. Klin. Med., 1888, xiv, 405. Ueber Diabetes mellitus.

MERING, I. Zeitschr. Klin. Med., 1889, xvi, 431. Ueber Diabetes mellitus.

MINKOWSKI, O. Arch. f. Exp. Path. u Pharm., 1893, xxxi, 85. Untersuchungen über den Diabetes mellitus nach Exstirpation des Pankreas.

MINKOWSKI, O. Pflüger's Arch., 1906, cxi, 13. Ueber die Zuckerbildung im organismus beim Pankreasdiabetes.

MORITZ, F. AND W. PRAUSNITZ. Zeitschr. f. Biol., 1890, xxvii, 81. Studien über den Phloridzindiabetes.

MÜLLER, F. AND J. SEEMANN. 1899, xiii, 209. Ueber die abspaltung von zucker aus Eiweiss.

NEBELTHAU, E. Muench. Med. Wochenschr., 1902, xlix, 917. Experimenteller Beiträg zur Lehre von der Zuckerbildung im diabetischen Organismus.

NEUBAUER, O. Deutsch. Arch. Klin. Med., 1909, xcv, 211. Über den abbau der Aminosäuren im gesunden und kranken Organismus.

NEUBERG, C. AND L. LANGSTEIN. Arch. f. Physiol., 1903, Suppl., 514. Ein Fall von Desamiderung im Thierkörper; zugleich ein Beiträg zur Frage nach der Herkunft des Glykogens.

NOTHMANN, M. Arch. f. exp. Path. u Pharm., 1925, cviii, 1. Über die Verteilung des Insulin im Organismus des normalen und pankreasdiabetischen Hundes.

OSBORNE, T. B. AND L. MENDEL. Journ. Biol. Chem., 1924, lix, 13. Nutrition and growth on diets highly deficient or entirely lacking in preformed carbohydrates.

PARNAS, J. K. AND R. WAGNER. Biochem. Zeitschr., 1922, cxxvii, 55. Beobachtungen über Zuckerneubildung. Nach Versuchen, die an einem Falle Besonderer Kohlenhydratstoffwechselstörung angestelltwurden.

PFLÜGER, E. Pflüger's Arch., 1892, li, 229. Ueber die Entstehung von Fett aus Eiweiss im Körper der Thiere.

PFLÜGER, E. Pflüger's Arch., 1903, xcvi, 1. Die Entdeckung des Glykogenes.

PFLÜGER, E. Pflüger's Arch., 1904, ciii, 1. Ueber die im thierischen Körper sich vollziehende Bildung von Zucker aus Eiweiss und Fett.

PFLÜGER, E. Pflüger's Arch., 1905, cvi, 168. Die Bedeutung der neuston Arbeiten über den Pankreas-Diabetes.

PFLÜGER, E. Pflüger's Arch., 1905, cviii, 115. Beiträg zur Frage nach dem Ursprung des im Pankreas-Diabetes ausgeschiedenen Zuckers.

PFLÜGER, E. AND P. JUNKERSDORF. Pflüger's Arch., 1910, cxxxi, 201. Ueber die Muttersubstanzes des glykogenes.

RAPPORT, D. AND E. P. RALLI. Amer. Journ. Physiol., 1928, lxxxv, 21. The nature of the food stuffs oxidized to provide energy in muscular exercise. II. In the phlorhizinized animal.

REILLY, F. H., F. W. NOLAN AND G. LUSK. Amer. Journ. Physiol., 1898, i, 395. Phlorhizin diabetes in dogs.

RINGER, A. I. AND G. LUSK. Zeitschr. f. Physiol. Chem., 1910, lxvi, 106. Über die Entstehung von Dextrose aus aminosäuren bei Phlorhizinglykosurie.

ROHMER, P. Zeitschr. f. Biol. 1910, liv, 455. Über Zuckerbildung aus verschiedenartigem Eiweiss.

ROSE, W. C. Journ. Biol. Chem., 1916, xxvi, 331. Protein feeding and creatine elimination in pancreatic diabetes.

ROSENFELD, G. Biochem. Zeitschr., 1912, xlii, 403. Über Glykogenbildung.

SCHÖNDORFF, B. Pflüger's Arch., 1900, lxxxii, 60. Über die Enstehung von glycogen aus Eiweiss.

SCHÖNDORFF, B. Pflüger's Arch. 1901, lxxxviii, 339. Die Entstehung von Glykogen aus Eiweiss.

SHAFFER, P. A. Journ. Biol. Chem., 1921, xlvii, 449. Antiketogenesis. II. The ketogenic antiketogenic balance in man.

SHAFFER, P. A. Journ. Biol. Chem., 1922, liv, 399. Antiketogenesis. IV. The ketogenic-antiketogenic balance in man and its significance in diabetes.

STILES, P. G. AND G. LUSK. Amer. Journ. Physiol., 1903, ix, 380. On the formation of dextrose in metabolism from the end-products of a pancreatic digest of meat.

WEISS, R. AND D. RAPPORT. Journ. Biol. Chem., 1924, lx, 513. The interrelations between certain amino acids and proteins with reference to their specific dynamic action.

WIERZUCHOWSKI, M. Journ. Biol. Chem., 1926, lxviii, 385. Respiratory metabolism in phlorhizin diabetes after glucose ingestion.

WILDER, R. M., W. M. BOOTHBY AND C. BELLER. Journ. Biol. Chem., 1922, li, 311. Studies of the metabolism of diabetes.

WILLIAMS, H. B., J. A. RICHE AND G. LUSK. Journ. Biol. Chem., 1912, xii, 349. Metabolism of the dog following the ingestion of meat in large quantity.

WILSON, R. H. AND H. P. LEWIS. Journ. Biol. Chem., 1930, lxxxv, 559. The formation of glycogen after oral administration of amino acids to white rats.

WOLFFBERG, S. Zeitschr. f. Biol., 1876, xii, 266. Ueber den Ursprung und die Aufspeicherung des Glykogens im thierischen Organismus.

CARBOHYDRATE TO FAT

BAUMGARDT, G. AND M. STEUBER. Deutsch. Arch. Klin. Med., 1920, cxxxiv, 241. Zur Frage der Fettbildung aus Kohlehydraten beim Menschen.

BLEIBTREU, M. Pflüger's Arch., 1901, lxxxv, 345. Fettmast und respiratorischer Quotient.

CHANIEWSKI, S. Zeitschr. f. Biol., 1884, xx, 179. Über Fettbildung aus Kohlehydraten im Thierorganismus.

ELLIS, N. R. AND O. G. HANKINS. Journ. Biol. Chem., 1925, lxvi, 101. Formation of fat in the pig on a ration moderately low in fat.

ELLIS, N. R. AND H. S. ISBELL. Journ. Biol. Chem., 1926, lxix, 239. The effect of food fat upon body fat as shown by the separation of the individual fatty acids of the body fat.

GRAFE, E. Deutsch. Arch. Klin. Med., 1914, cxiii, 1. Die Wirkung einer längeren, irberreichlichen Kohlehydratkost ohne Eiweiss auf den Stoffwechsel von Mensch und Tier.

HANRIOT, M. Compt. Rend. Acad. Sci., 1892, cxiv, 371. Sur l'assimilation des hydrates de carbone.

HOPPE-SEYLER, F. Zeitschr. f. physiol. Chem., 1879, iii, 351. Ueber-Gärungsprozesse.

HOFFMAN, A. AND E. WERTHEIMER. Pflüger's Arch., 1927, ccxvii, 728. Zur Physiologie des Fettgewebes und der Fettablagerung.

KROGH, A. AND J. LINDHARD. Biochem. Journ., 1920, xiv, 290. The relative value of fat and carbohydrate as sources of muscular energy.

LEATHES, J. B. Problems in animal metabolism. London, 1906.

LEHMANN, K. B. AND E. VOIT. Zeitschr. f. Biol., 1901, xlii, 619. Die Fettbildung aus Kohlehydraten.

LUBLIN, A. Arch. f. exp. Path. u Pharm., 1926, cxv, 101. Der Einfluss des Insulins auf die Kohlenhydratverwertung im nichtdiabetischen Organismus.

LUBLIN, A. Arch. f. exp. Path. u Pharm., 1927, cxxv, 229. Das Verhalten des respiratorischen Stoffwechsels nach Kohlenhydratdarreichung unter dem Einfluss von Adrenalin, etc.

LUSK, G. Journ. Biol. Chem., 1915, xx, 555. An investigation into the causes of the specific dynamic action of the foodstuffs.

MAGNUS-LEVY, A. Pflüger's Arch., 1894, lv, 1. Ueber die grösse des respiratorischen Gaswechsels unter dem Einfluss der Nahrungsaufnahme.

MAGNUS-LEVY, A. Arch. f. exp. Path. u. Pharm., 1899, xlii, 149. Die oxybuttersäure und ihre Beziehungen zum Coma diabeticum.

MEISSL, E. Zeitschr. f. Biol., 1886, xxii, 63. Untersuchungen über den Stoffwechsel des Schweines.

MEISSL, E. AND F. STROHMER. Sitzungsber d. k. Akad. der Wissensch., 1883, lxxxviii, 3 abth. Quoted by MEISSEL, 1886.

MORGULIS, S. AND J. H. PRATT. Amer. Journ. Physiol., 1913, xxxii, 200. On the formation of fat from carbohydrates.

MUNK, I. Arch. f. path. Anat., 1885, ci, 91. Die Fettbildung aus Kohlehydraten beim Hunde.

NENCKI, M. Journ. Prakt. Chem., 1878, xvii, 105. Quoted by LEATHES AND RAPER, The fats.

NEUBERG, C. AND B. ARINSTEIN. Biochem. Zeitschr., 1921, cxvii, 269. Vom Wesen der Buttersäure und Butylalkoholgärung.

PEMBREY, M. S. Journ. Physiol., 1901–02, xxvii, 407. The respiratory exchange during the deposition of fat.

RAPPORT, D. AND E. P. RALLI. Amer. Journ. Physiol., 1928, lxxxiii, 450. The nature of the foostuffs oxidized to provide energy in muscular exercise. I. In the normal animal.

RAPPORT, D., R. WEISS AND F. A. CSONKA. Journ. Biol. Chem., 1924, lx, 583. The respiratory metabolism of a young pig as influenced by food and benzoic acid.

RAPER, H. S. Journ. Chem. Soc., 1907, xci, 1831. The condensation of acetaldehyde and its relation to the biochemical synthesis of fatty acids.

REISET, J. Ann. de Chim. et de Phys. (3rd Series), 1863, lxix, 129. Recherches chimiques sur la respiration des animaux d'une ferme.

REGNAULT, V. AND J. REISET. Ann. de Chim. et de Phys. (3rd Series), 1849, xxvi, 299. Recherches chimique sur la respiration des animaux des diverses classes.

RUBNER, M. Zeitschr. f. Biol., 1886, xxii, 272. Ueber die Fettbildung aus Kohlehydraten im Körper des Fleischfressers.

SCHULTZE, B. Landw. Jahrb., 1882, i, 57. Ueber Fettbildung im Thierkörper.

SMEDLEY, I. Journ. Physiol., 1912, xlv, Proc. p. 25. The biochemical synthesis of fatty acids from carbohydrate.

SMEDLEY, I. AND E. LUBRZYNSKA. Biochem. Journ., 1913, vii, 364. The biochemical synthesis of fatty acids.

SOXHLET, O. Zeitschr. d. Landw. Verein. in Bayern., Aug. 1881. Quoted by BLEIBTREU, Pflüger's Arch., 1901, lxxxv, 345.

TSCHIRWINSKY, N. Landw. Versuchstat., 1883, xxix, 317. Zur Frage über die Fettbildung im Thierischen organismus.

VOIT, C. Hermann's Handbch. d. Physiol., Leipzig, 1881.

WESSON, L. G. Journ. Biol. Chem., 1927, lxxiii, 507. A fat formation under abnormal conditions from carbohydrate by the rat, and its relationship to a possible new dietary factor.

WIERZUCHOWSKI, M. AND S. M. LING. Journ. Biol. Chem., 1925, lxiv, 697. On fat production in a young hog.

FAT TO CARBOHYDRATE

ABDERHALDEN, E. AND P. RONA. Zeitschr. f. Physiol. Chem., 1904, xli, 303. Bildung von Zucker aus Fett.

ALLARD, E. Arch. exp. Path. u Pharm., 1908, lix, 111. Über die Beziehungen der Umgebungstemperatur zur Zuckerausscheidung beim Pankreasdiabetes.

ANDERSON, R. J. AND G. LUSK. Journ. Biol. Chem., 1917, xxxii, 421. The interrelation between diet and body condition and the energy production during mechanical work.

ATWATER, W. O. AND F. G. BENEDICT. U. S. Dept. Agric., Exp. Station Bull., cxxxvi, 1903. Metabolism of matter and energy in the human body.

BENEDICT, F. G. AND R. D. MILNER. U. S. Dept. Agric., Exp. Station Bull. clxxv, 1907. Metabolism of matter and energy in the human body.

BENEDICT, F. G. AND E. P. JOSLIN. Carn. Inst. of Wash., Publ. cxxxvi, 1910. Metabolism in diabetes mellitus.

BENEDICT, F. G. AND E. P. JOSLIN. Carn. Inst. of Wash., Publ. clxxvi, 1912. A study of the metabolism in severe diabetes.

BENEDICT, F. G. AND E. P. CATHCART. Carn. Inst. of Wash., Publ. clxxxvii, 1913. Muscular work.

BERNSTEIN, S. AND W. FALTA. Deutsch. Arch. f. Klin. Med., 1918, cxxvii, 1. Besteht beim Diabetes mellitus eine Steigerung der Zuckerbildung oder eine Störung des Zuckerverbrauches?

BLUM, F. Pflüger's Arch., 1902, xc, 617. Weitere Mittheilungen zur Lehre von dem Nebennierendiabetes.

BEST, C. H., K. FURUSAWA AND J. H. RIDOUT. Proc. Roy. Soc. (B), 1929, civ, 119. The respiratory quotient of the excess metabolism of exercise.

BOCK, A. V., C. VANCAULAERT AND D. B. DILL. Journ. Physiol., 1928, lxvi, 136. The "steady state" and the respiratory quotient during work.

BOCK, A. V., C. VANCAULAERT AND D. B. DILL. Journ. Physiol., 1928, lxvi,162. The "steady state" and the respiratory quotient during work.

BODEY, M. G., H. B. LEWIS AND J. F. HUBER. Journ. Biol. Chem., 1927, lxxv, 715. The absorption and utilization of insulin as evidenced by glycogen formation in the white rat.

BOLLMAN, J. T., F. C. MANN AND T. B. MAGATH. Amer. Journ. Physiol., 1925, lxxiv, 238. Muscle glycogen following total removal of the liver.

BORNSTEIN, A. AND W. GRIESBACH. Zeitschr. ges. exp. Med., 1923, xxxvii, 33. Über Zuckerverbrennung in der künstlich durchbluteten Leber.

BURN, J. H. AND H. H. DALE. Journ. Physiol., 1924, lix, 164. On the location and nature of the action of insulin.

BURN, J. H. AND H. P. MARKS. Journ. Physiol., 1926, lxi, 497. The production of sugar in the perfused liver from non-protein sources.

CALVO-CRIADO, V. Biochem. Zeitschr., 1925, clxiv, 76. Nachweis der Entstehung von Kohlehydraten aus Fett und Abhängigkeit derselben von der Leber.

CAMPBELL, W. R. AND J. MARKOWITZ. Amer. Journ. Physiol., 1927, lxxx, 548. The fate of dihydroxyacetone in the animal body.

CAMPOS, F. A., W. B. CANNON, H. LUNDIN AND T. T. WALKER. Amer. Journ. Physiol., 1929, lxxxvii, 680. Some conditions affecting the capacity for prolonged muscular work.

CATHCART, E. P. AND J. MARKOWITZ. Journ. Physiol., 1927, lxiii, 309. The influence of various sugars on the respiratory quotient.

CHAIKOFF, I. L. Journ. Biol. Chem., 1927, lxxiv, 203. Observations on the ketone body excretion, the dextrose to nitrogen ratios, and the glycogen content of liver and muscles of fasted, depancreatized dogs.

CHAIKOFF, I. L., J. J. R. MACLEOD, J. MARKOWITZ AND W. W. SIMPSON. Amer. Journ. Physiol., 1925, lxxiv, 36. Observations on depancreatized dogs before and after withdrawal of insulin.

CHAIKOFF, I. L. AND J. J. WEBER. Journ. Biol. Chem., 1928, lxxvi, 813. The formation of sugar from fatty acids in the depancreatized dog injected with epinephrin.

CHAIKOFF, I. L. AND J. J. R. MACLEOD. Quart. Journ. exp. Physiol., 1929, xix, 291. The effect of shivering on the respiratory quotient in pancreatic diabetes.

CHAUVEAU, A. Compt. Rend. Acad. Sci., 1896, cxxii, 1163. Source et nature du potential directement utilise dans le travail musculare, d'apres les echanges respiratoires, chez l'homme en état d'abstinence.

CHAUVEAU, A. Compt. Rend. Acad. Sci., 1897, cxxv, 1070. Comparaison du pouvoir thermogène ou dynamogène des elements simples avec leur pouvoir nutritif.

CHAUVEAU, A. Compt. Rend. Acad. Sci., 1898, cxxvi, 795. Sur l'importance du sucre considéré comme aliment, etc.

CHAUVEAU, A. Compt. Rend. Acad. Sci., 1898, cxxvi, 1072. La viande et l'amidon comparés au sucre, au point de vue de la valeur nutritive, chez le sujet que travaille.

CHAUVEAU, A. Compt. Rend. Acad. Sci., 1898, cxxvi, 1118. Le sucre et la graisse, au point de vue de leur valeur nutritive respective, chez le sujet constamment tenu au repas, etc.

CLARK, A. H. Journ. Exp. Med., 1916, xxiv, 621. The interrelation of the surviving heart and pancreas of the dog in sugar metabolism.

COLLENS, W. S., D. H. SHELLING AND C. S. BYRON. Amer. Journ. Physiol.,1927, lxxix, 689. Effect of adrenalin upon blood sugar following ligation of the hepatic artery.

CORI, C. F. AND G. T. CORI. Journ. Biol. Chem., 1928, lxxix, 309. The influence of epinephrin on the carbohydrate metabolism of fasting rats, with a note on new formation of carbohydrates.

CORI, C. F. AND G. T. CORI. Journ. Biol. Chem., 1928, lxxix, 321. The influence of epinephrin and insulin on the carbohydrate metabolism of rats in the postabsorptive state.

CORI, C. F. AND G. T. CORI. Journ. Biol. Chem., 1928, lxxix, 343. The influence of epinephrin on the utilization of absorbed glucose.

CORI, C. F. AND G. T. CORI. Journ. Biol. Chem., 1928, lxxxiv, 683. The influence of epinephrin on lactic acid production and blood sugar utilization.

CORI, C. F. AND G. T. CORI. Journ. Biol. Chem., 1929, lxxxv, 275. The influence of insulin and epinephrin on glycogen formation in the liver.

CSONKA, F. A. Journ. Biol. Chem., 1916, xxvi, 93. The influence of ingested carbohydrate, protein and fat on the blood sugar in phlorhizin diabetes.

DEUEL, H. J., H. E. C. WILSON AND A. T. MILHORAT. Journ. Biol. Chem., 1927, lxxiv, 265. On the mechanism of phlorhizin diabetes.

DOISY, E. A. AND J. W. BECKMANN. Journ. Biol. Chem., 1922, liv, 683. The relation existing between arterial and venous blood of the dog with special reference to the plasma chloride.

EMBDEN, G. Beitr. z. chem. Physiol. u Path., 1905, vi, 44. Über Zuckerbildung bei künstlicher Durchblutung der glykogenfreien Leber.

EMBDEN, G. AND H. SALOMON. Beitr. z. chem. Physiol. u Path., 1905, vi, 63. Fütterungsversuche am pankreaslosen Hunde.

EPPINGER, H., W. FALTA AND C. RUDINGER. Zeitschr. f. Klin. Med., 1908, lxvi, 1. Ueber die Wechselwirkungen der Drüsen mit innerer Sekretion.

FALTA, W., F. GROTE AND R. STAEHELIN. Beit. z. chem. Physiol. u Path., 1907, x, 199. Versuche über Stoffwechsel und Energieverbrauch an pankreaslosen Hunden.

FRENTZEL, J. AND F. REACH. Pflüger's Arch., 1901, lxxxiii, 477. Untersuchungen zur Frage nach der quelle der Muskelkraft.

FURUSAWA, K. Proc. Roy. Soc. (B) 1925, xcviii, 287. Muscular exercise, lactic acid, and the supply and utilisation of oxygen.

GEELMUYDEN, H. S. Skand. Arch. Physiol., 1920, xl, 211. Über das Schicksal der Ketonkörper im intermediaren Stoffwechsel und über Zuckerbildung aus Fett.

GEELMUYDEN, H. C. Ergebn. Physiol., 1925, xxiv, 1. Über die spezifesch-dynamische Wirkung der Nahrungsstoffe und ihre Beziehungen zum grundumsatz beim Diabetes Mellitus.

GEYELIN, H. R. AND E. F. DUBOIS. Journ. Amer. Med. Assoc., 1916, lxvi, 1532. A case of diabetes of maximum severity with marked improvement.

GRAFE, E. AND H. SALOMON. Deutsch. Arch. f. Klin. Med., 1922, cxxxix, 369. Über den Einfluss der Muskelarbeit auf die Intensität der Zuckerverbrennung beim Diabetiker.

HALSEY, J. T. Sitzungsber der Gesellsch. zur Beförderungen der gesammt wissensch. zu Marburg, nr. 5, 1899. (Cited by P. JUNKERSDORF, Pflüger's Arch., 1911, cxxxvii, 269.)

HANISCH, S. AND P. JUNKERSDORF. Pflüger's Arch., 1927, ccxvii, 264. Das Stoffwechselgeschehen im Phlorihizindiabetes bei einseitiger Ernährung mit Eiweiss.

HARI, P. Pflüger's Arch., 1909, cxxx, 112. Der respiratorische gaswechsel der winterschlafenden Fledermaus.

HÉDON, L. Arch. Intern. Physiol., 1926, xxvii, 354. Le quotient resperatoire et le metabolisme de base dans le diabete pancreatique experimental.

HEINEMANN, H. N. Pflüger's Arch., 1901, lxxxiii, 441. Experimentelle untersuchung am Menschen über den Einfluss der Muskelarbeit auf den Stoffverbrauch und die Bedeutung der einzelnen Nährstoffe als Quelle der Muskelkraft.

HENDERSON, Y. AND H. W. HAGGARD. Amer. Journ. Physiol., 1925, lxxii, 264. The maximum of human power and its fuel.

HETZEL, K. S. AND C. N. H. LONG. Proc. Roy. Soc., 1926 (B) xcix, 279. The metabolism of the diabetic individual during and after muscular exercise.

HILDEBRANDT, F. Arch. f. exp. Path. u Pharm., 1920, lxxxviii, 80. Über die Bezuhungen Zwischen Hyperglykämie Adrenalindiabetes.

HILL, A. V., C. N. H. LONG AND H. LUPTON. Proc. Roy. Soc. (B) 1924, xcvi, 455. Muscular exercise, lactic acid and the supply and utilization of oxygen. III.

HIMWICH, H. E. AND W. B. CASTLE. Amer. Journ. Physiol., 1927, lxxxiii, 92. The respiratory quotient of resting muscle.

HIMWICH, H. E. AND M. I. ROSE. Amer. Journ. Physiol., 1929, lxxxviii, 663. The respiratory quotient of exercising muscle.

KNOWLTON AND E. H. STARLING. Journ. Physiol., 1912, xlv, 146. Experiments on the consumption of sugar in the normal and diabetic heart.

KOJIMA, Y. Biochem. Zeitschr., 1927, cxc, 352. Fortgestzte Untersuchungen zur Frage der Enstehung von Zucker aus Fett.

KOJIMA, Y. Biochem. Zeitschr., 1928, cxcvii, 31. Fortgesetzte Untersuchungen zur Frage der Enstehung von Zucker aus Fett.

KRAMER, B., J. MARKER AND J. R. MURLIN. Journ. Biol. Chem., 1916, xxvii, 499. Is the glucose retained when sodium carbonate is administered to depancreatized dogs deposited as glycogen?

KROGH, A. AND J. LINDHARD. Biochem. Journ., 1920, xiv, 290. Relative values of fat and carbohydrate as sources of muscular energy.

LAFON, G. Recherches experimentale sur le diabète et sur la glycogénie. (These doct. toulouse, 1906) Cited by HÉDON, Arch. Intern. Physiol., 1926, xxvii, 254.

LAUFBERGER, W. Klin. Wochenschr., 1924, iii (1), 264. Theorie der Insulinwirkung.

LEATHES, J. B. Problems in animal metabolism. London, 1906.

LESSER, E. J. Biochem. Zeitschr., 1920, ciii, 1. Über das Wesen des Pankreasdiabetes.

LESSER, E. J. AND K. ZIPF. Biochem. Zeitschr., 1923, cxl, 435. Sind pankreasdiabetische Lebern adrenalinempfindlich?

LOEWI, O. Arch. f. exp. Path. u. Pharm., 1902, xlvii, 68. Zur Frage nach der Bildung von Zucker aus Fett.

LUSK, F. Zeitschr. f. Biol., 1901, xlii, 31. Über Phlorhizindiabetes.

LUSK, G. Amer. Journ. Physiol., 1908, xxii, 163. The production of sugar from glutamic acid ingested in phorhizin glycosuria.

LUSK, G. Arch. Int. Med., 1915, xv, 939. On the diabetic respiratory quotient.

MACLEAN, H. AND I. SMEDLEY. Journ. Physiol., 1913, xlv, 470. The behaviour of the diabetic heart towards sugar.

MACLEOD, J. J. R. AND R. G. PEARCE. Amer. Journ. Physiol., 1913, xxxii, 184. The sugar consumption in normal and diabetic dogs after evisceration.

MACLEOD, J. J. R. AND R. G. PEARCE. Amer. Journ. Physiol., 1914, xxxiii, 378. Further observations on the rate at which sugar disappears from the blood of eviscerated animals.

MACLEOD, J. J. R. AND J. MARKOWITZ. Trans. Assoc. Amer. Phys., 1926, xli, 147. Further researches on depancreatized animals treated with insulin.

MAGNUS-LEVY, A. Arch. f. Physiol., 1904, p. 377. Ueber Zuckerbildung aus Eiweiss und das Verhalten des respiratorischen Quotienten im Diabetes.

MANN, F. C. AND T. B. MAGATH. Arch. Int. Med., 1923, xxxi, 797. The effect of total removal of the liver, after pancreatectomy, on the blood sugar level.

MANN, F. C. Ergebn. Physiol., 1925, xxiv, 379. The extra-hepatic formation of bilirubin.

MARKOWITZ, J. Amer. Journ. Physiol., 1925, lxxiv, 22. Glyconeogenesis.

MARKOWITZ, J. Amer. Journ. Physiol., 1928, lxxxiii, 698. The respiratory exchange of diabetic and non-diabetic dogs following hepatectomy.

MARSH, M. E. Journ. Nutr., 1928, i, 57. The character of energy metabolism during work.

MERING, I. Zeitschr. f. Klin. Med., 1888, xiv, 405. Ueber Diabetes Mellitus.

MERING, I. Zeitschr. f. Klin. Med., 1889, xvi, 431. Ueber Diabetes Mellitus.

MCMASTER, P. D. AND D. R. DRURY. Proc. Soc. Exp. Biol. and Med., 1927, xxv, 151. Respiratory quotient in conditions of liver insufficiency.

MEYERHOF, O. Pflüger's Arch., 1919, clxxv, 20. Über die Atmung der Froschmuskulatur.

MEYERHOF, O. AND H. E. HIMWICH. Pflüger's Arch., 1924, ccv, 415. Beiträge zum Kohlehydratstoffwechsel des warmblütermuskels, inbesondere- nach einseitiger Fetternährung.

MINKOWSKI, O. Arch. f. exp. Path. u Pharm., 1887, xxiii, 142. Über experimentelle glykosurie bei Vögeln.

MINKOWSKI, O. Pflüger's Arch., 1906, cxi, 13. Über die Zuckerbildung im Organismus beim Pankreasdiabetes.

MOHR, L. Zeitschr. f. exp. Path. u. Therap., 1907, iv, 910. Untersuchungen über den Diabetes Mellitus.

MOORHOUSE, V. H. K., S. W. PATTERSON AND M. STEPHENSON. Biochem. Journ., 1815, ix, 171. A study of the metabolism in experimental diabetes.

MORTIZ, F. AND W. PRAUSNITZ. Zeitschr. f. Biol., 1890, xxvii, 81. Studien über den Phlorhidzindiabetes.

MURLIN, J. R., L. EDELMANN AND B. KRAMER. Journ. Biol. Chem., 1913, xvi, 79. The carbon dioxide and oxygen content of the blood after clamping the abdominal aorta and inferior vena carva below the diaphragm.

NAGAI, H. Zeitschr. f. allg. Physiol., 1909, ix, 243. Der Stoffwechsel des Winterschläfers.

PALAZZOLO, G. Arch. di fisiol., 1912–13, xi, 558. Ricerche sul consumo del grasso muscalare durante la contrazione.

PARNAS, J. K. AND R. WAGNER. Biochem. Zeitschr., 1922, cxxvii, 55. Beobachtungen über Zuckerneubildung.

PATTERSON, S. W. AND E. H. STARLING. Journ. Physiol., 1913, xlvii, 137. The carbohydrate metabolism of the isolated heart-lung preparation.

PAULESCO, N. Compt. Rend. Soc. Biol., 1913, lxxv, 588. Origines du glycogène. Acides gras, glycerine, alcool éthylique.

PAVY, E. W., T. G. BRODIE AND R. L. SIAU. Journ. Physiol., 1903, xxix, 467. On the mechanism of phlorhidzin glycosuria.

PEMBREY, M. S. Journ. Physiol., 1901, xxvii, 66. Observations upon the respiration and the temperature of the marmot.

PESERICO, E. Arch. di fisiol., 1925, xxiii, 488. L'Azione della insulina sul ricambio respiratorio del cuore isolato di mammifero.

PFLÜGER, E. Pflüger's Arch., 1907, cxix, 117. Über den Einfluss einseitiger Ernährung oder Nahrungsmangels auf den glykogenanhalt des thierischen Körpers.

PFLÜGER, E. AND P. JUNKERSDORFF. Pflüger's Arch., 1910, cxxxi, 201. Über die Muttersubstanzen des glykogenes.

POLLACK, L. Biochem. Zeitschr., 1922, cxxvii, 120. Über den Eingluss von Aminosaüren und Fettsäuren auf die Blutzuckerregulation.

PORGES, O. Biochem. Zeitschr., 1910, xxvii, 131. Über den respiratorischen Quotienten nach Ausschaltung der Abdominalorgane.

PORGES, O. AND H. SALOMON. Biochem. Zeitschr., 1910, xxvii, 143. Über den respiratorischen Quotienten pankreas-diabetischen Hunde nach ausschaltung der Abdominalorgane.

RABINOWITCH, I. Journ. Clin. Invest., 1925, ii, 143. Simultaneous respiratory exchange and blood sugar curves, etc.

RAPPORT, D. Amer. Journ. Physiol., 1929, xci, 238. The utilization of the "waste heat" of metabolism in muscular exercise.

RAPPORT, D. AND E. P. RALLI. Amer. Journ. Physiol., 1928, lxxxiii, 450. The nature of the foodstuffs oxidized to provide energy and muscular exercise. I. In the normal animal.

RAPPORT, D. AND E. P. RALLI. Amer. Journ. Physiol., 1928, lxxxv, 21. The nature of the foodstuffs oxidized to provide energy and muscular exercise. II. In the phlorhizinized animal.

REGNAULT, V. AND J. REISET. Ann. de chim. et de phys. (3rd. series), 1849, xxvi, 299. Recherches chimique sur la respiration des animaux des diverises classes.

RICHARDSON, H. B. Physiol. Rev., 1929, ix, 61. The respiratory quotient.

RICHARDSON, H. B. AND S. Z. LEVINE. Journ. Biol., Chem., 1925, lxvi, 161. Exercise and the respiratory quotient in diabetes.

RICHARDSON, H. B., R. D. LOEBEL AND E. SHORR. Proc. Soc. Exp. Biol. and Med., 1926–27, xxiv, 243. The metabolism and the respiratory quotient of excised renal tissue.

RICHARDSON, H. B., R. D. LOEBEL AND E. SHORR. Proc. Soc. Exp. Biol. and Med., 1928, xxv, 658. Respiratory metabolism of excised normal and diabetic tissue.

RINGER, A. I. Journ. Exp. Med., 1910, xii, 105. The influence of adrenalin in phlorhizin diabetes.

RINGER, A. I. Journ. Biol. Chem., 1912, xii, 431. Protein metabolism in experimental diabetes.

RINGER, A. I. Journ. Biol. Chem., 1912, xii, 511. The quantitative conversion of propionic acid into glucose.

RINGER, A. I. AND G. LUSK. Zeitschr. physiol. Chem., 1910, lxvi, 106. Über die Entstehung von Dextrose aus Aminosäuren bei Phlorhizinglykosurie.

RITZMANN, H. Arch. f. exp. Path. u Pharm., 1909, lxi, 231. Über den Mechanismus der Adrenalinglykosurie.

ROLLY, F. Deutsch. Arch. Klin. Med., 1912, cv, 494. Zur Theorie und Therapie des Diabetes Mellitus.

ROSENFELD, S. Verhandl. d. Kongr. f. Inn. Med., 1893, xii, 366. Ueber Phlorhidzinwirkungen.

ROSENFELD, G. Biochem. Zeitschr., 1912, xlii, 403. Über glycogenbildung.

ROUBITSCHEK, R. Pflüger's Arch., 1914, clv, 68. Zur Frage der Zuckerbildung aus Fett.

SANSUM W. D. AND R. T. WOODYATT. Journ. Biol. Chem., 1915, xxi, 1. A study of narcotic drugs in phlorhizin diabetes.

SEEGEN, J. Pflüger's Arch., 1886, xxxix, 132. Über die Fähigkeit der Leber, Zucker aus Fett zu bilden.

SOSKIN, S. Amer. Journ. Physiol., 1927, lxxxi, 382. Muscle glycogen as a source of blood sugar.

STARLING, E. H. AND C. L. EVANS. Journ. Physiol., 1914, xlix, 67. The respiratory exchange of the heart in the diabetic animal.

TAKANE, R. Biochem. Zeitschr., 1926, clxxi, 403. Athmung und Koblehydratumsatz in Leber und Muskel des Warmblüters.

TAKAO, T. Biochem. Zeitschr., 1926, clxxii, 272. Über einige Versuche zur Frage der Beeinflussung des Kohlehydratstoffwechsels durch Fettzufuhr.

VERZÁR, F. Biochem., Zeitschr., 1914, lxvi, 75. Die Verbrennung des Zuckers im Pankreasdiabetes.

VERZÁR, F. AND A. V. FEJER. Biochem. Zeitschr., 1913, liii, 140. Die Berbrennung von Traubenzucker im Pankreasdiabetes.

VERZÁR, F. AND J. KRAUSS. Biochem. Zeitschr., 1914, lxvi, 48. Der Zuckerverbrauch des überlebenden Darmes von pankreasdiabetschen Hunden.

VISSCHER, M. B. Amer. Journ. Physiol., 1928, lxxxv, 410. On the sources of energy for cardiac contraction.

WEISS, J. Zeitschr. f. physiol. Chem., 1898, xxiv, 542. Über die Bildung von Zucker aus Fett im Thierkörper.

WERTHEIMER, E. Pflüger's Arch., 1926, ccxiii, 298. Die inkretorische Beeinflussung der Umwandlung von Fett im Kohlenhydrate im der Leber.

WIERZUCHOWSKI, M. Journ. Biol. Chem., 1926, lxviii, 385. Respiratory metabolism in phlorhizin diabetes after glucose ingestion.

WIERZUCHOWSKI, M. Journ. Biol. Chem., 1927, lxxiii, 417. Intermediary carbohydrate metabolism. II. Ketosis in phlorhizin diabetes. III. Vital action of glucose in phlorhizin diabetes.

WILDER, R. M., W. M. BOOTHBY AND C. BEELER. Journ. Biol. Chem., 1922, li, 311. Studies on the metabolism of diabetes.

WILSON, J. R., S. Z. LEVINE, H. RIVKIN AND F. B. BERLINER. Amer. Journ. Dis. Child., 1928, xxxiii, 618. Respiratory metabolism in infancy and in childhood.

WINFIELD, G. Journ. Physiol., 1915, xlix, 171. The fate of fatty acids in the survival proccesses of muscle.

ZUNTZ, N. Oppenheimer's Handbch. der Biochemie, Jena, 1911, iv, 857. Betrachtungen über die Beziehungen zwischen Nahrstoffen und Leistungen des Körpers.

THE RÔLE OF THE SMALL INTESTINE IN NUTRITION

H. E. MAGEE

The Rowett Research Institute, Aberdeen, Scotland

The title chosen for this article requires some further definition. A rigid delimitation is, however, neither possible nor desirable.

The small intestine is the portal by which practically all the nutritive material enters the body. Absorption is its predominant rôle, digestion and the onward movement of food being preliminary stages of this process. These considerations determine the nature of this review and, while attention is mainly given to the problems of absorption, those of the digestive and motor functions of the intestine will not be disregarded.

The subject of absorption may be approached from two aspects. First, there are the fundamental problems concerning the processes that determine the passage of water and soluble digestion products from the intestinal lumen into the circulating body fluids; and secondly, the influences, proximate and remote, of the nature of the food ingested on the functions of the small intestine. Regard must also be paid to other organs and to physiological states of the body as a whole that may have definite influences on the assimilation of food.

ABSORPTION. The subject of intestinal absorption has already been reviewed in this journal by Goldschmidt (1). The general impression obtained from his article is that the laws of physics and chemistry, although inadequate to explain all the phenomena connected with absorption, are nevertheless factors of very considerable importance. He expressed the opinion that further study of the subject would very probably demonstrate greater and greater dependence of the absorptive processes on physical laws as then established.

Has the position altered appreciably since then? The volume of experimental work done with a direct bearing on the subject has not been as great as its importance deserves; but the evidence that has accumulated has tended to place a greater degree of responsibility on the activity of the epithelial cells of the villi. Thus in 1924, Höber (2), one of the principal antagonists of the "selective action" view of absorption, stated that no doubt could exist as to the presence of a specific motive force in the intestinal epithelial cells.

The laws of physics and chemistry, in so far as they can be considered relevant to the subject, have not undergone significant alteration in the past eight years, so that the means available for elucidating the problems of absorption are essentially the same as when Goldschmidt's review was written. On the other hand, the opinions of Verzár (3), recently published, suggest a revival of the old controversy in a somewhat modified form. He states that absorption is essentially a passage of substances, soluble in water or in lipoids, through a complicated colloidal membrane, whose permeability can be altered by intrinsic metabolic processes and extrinsic factors, and that the forces operating are the same as hold good for the movements of fluid through dead membranes. It will, therefore, be necessary, in discussing this question, to review again much of the literature covered by Goldschmidt.

METHODS OF INVESTIGATION. As the inferences to be drawn from experimental work depend to a very large extent on the methods used, a brief discussion of these is desirable.

1. Loops of intestine in anesthetised or decerebrate animals: In this method one or more loops are separated from the small intestine leaving the mesenteric connections intact, one loop being generally used as a control. The substance under consideration is placed in a loop previously washed out. The open ends are closed and the loop replaced in the abdomen. After an interval, the contents are expelled, analysed and the rate of absorption of the substance determined by difference. This method has been used by Hamburger, Heidenhain, Cohnheim, Reid, Höber and many others. More recently Hewitt (4), and Edkins and Murray (5), (6) have used it in modified form on decapitated or decerebrated cats. Hédon (7), believing that anesthetics could influence the results, applied the method to unanesthetised rabbits.

The method has several drawbacks of which the chief is the great difficulty in recovering all the contents of the loop at the conclusion of the experiment. Cobet (8), after finding considerable variations in the amounts of fluid recovered from intestinal loops into which equal amounts were placed, has outlined a procedure by which this difficulty can be overcome.

2. Thiry-Vella fistulae: This method and numerous modifications have been used very extensively for studies of absorption and of the succus entericus (Babkin (9)). Recently, White and Rabinovitch (10) have employed the technique in experiments with glucose solutions, and Walsh and Ivy (11) for experiments on the locus of excretion of calcium. The procedure is practically the same as under 1, but the sources of

error are greater because of the increased difficulty in emptying the loop completely. Furthermore, after successive experiments a catarrhal condition is liable to develop with an excessive secretion of juice (12). Another disadvantage is that before the animal can be used for experiments some time must elapse during which the loop has not functioned; so that degenerative changes in the mucosa are probable, as will be seen later.

3. The polyfistula method devised by London (13): In this, fistulae of the intestine at different levels are made through which samples of chyme can be drawn off and analysed. London (14) has also used the method to determine the rate of absorption of nitrogen from protein digests, introduced through one of the fistulae by means of a special double cannula, the intestine being closed off by means of a balloon. Even with these devices the sources of error would appear to be greater than with Thiry-Vella loops. Results obtained without his special apparatus are a measure, not only of the rate of absorption, but also of the digestive and motor functions of the intestine. The method has been used recently by Never (15).

4. Artificial perfusion of the intestine with physiological solutions or defibrinated blood: The method has been used only to a very limited extent; by Cohnheim (16) on the intestine of dogs at variable times *post mortem.* Recently, Mond (17) has used it on living frog's intestine.

5. The method of Cori (18) (19): Rats from 120 to 180 grams weight, fasted for at least 24 hours, are fed concentrated solutions of sugars or amino-acids by stomach tube. They are then killed after a suitable interval, the entire gastro-intestinal tract excised and the unabsorbed residue determined. From the amount of substance which disappears the "absorption coefficient," or the number of grams absorbed per 100 grams weight of rat per hour, is calculated. The method is the most physiological of all and has been used successfully not only by Cori and his associates but also by Pierce, Osgood and Polansky (20) (21), Kokas and Gal (22) and others. The results can, however, be affected by variations in the state of the mucosa and in the motor functions of the stomach and intestine. Thus, Cori (19) has reported diarrhea as a frequent occurrence and both he and Auchinachie, Macleod and Magee (23) have frequently observed little or no absorption to take place, perhaps because in the former case, a catarrhal condition was established and, in the latter, spasm of the pyloric sphincter. As a means of studying the rate of absorption of simple substances, singly or mixed with others, Cori's method offers a wide field for experimentation; but neither it

nor any of the above procedures can throw any very direct light on the rôles played by the different structures in the gut-wall on the absorptive processes. In all these methods the circulating fluids, the nervous and hormonal influences are complicating factors that cannot be eliminated, in so far as one can see at present. An attempt has been made by Körösy (24) to eliminate some of these influences by means of a heart-lung-intestine circulation. The method does not appear to have been used by any other observers.

In the foregoing methods it is the unabsorbed residue that is estimated, so that the rate of absorption is measured indirectly.

6. Surviving segments: With surviving intestinal loops freed from mesentery and suspended in oxygenated Tyrode's or Locke's solution the complications just mentioned are eliminated and the experimental conditions can be adequately controlled. The method has the advantage that the rate of passage of solutes from the lumen can be measured directly, and possible changes in their constitution more readily determined in the protein-free outer fluid, than when they are absorbed into the blood.

The method suffers from the disadvantage that the path available for the passage of molecules from the inner to the outer fluid is directly through the submucous, muscular and serous coats instead of into the circulating blood and lymph. Further, as Verzár and Kokas (25) have found that the rhythmical contractions of the villi come to an end with the interruption of the blood supply, this factor must also be in abeyance. As is the case in all methods on isolated organs, the tissues during the experiments are in a dying state but, in spite of these limitations, the method has afforded valuable results in the hands of numerous observers, both early and recent.

7. A modification of a procedure used for digestibility trials in farm animals has recently been reported by Bergeim (26). The underlying principle is to feed a quantity of an unabsorbable substance, such as chromium, and, from the ratio of this element to the other constituents of the ingesta and feces, the utilisation of different materials can be determined. To give accurate results the foreign supplement should be unabsorbable and should be devoid of physiological action. Bergeim uses relatively large doses of iron salts which fulfill neither condition, and it is questionable if even chromium does, in view of the striking effects attributed by Hart, Steenbock *et al.* (27) to the ingestion of traces of copper.

The method has limitations similar to the polyfistula procedures, as

digestion and movements are complications. It is, however, very probable that a combination of both techniques, as suggested by Bergeim (28) would, if well controlled, throw much light on the normal mechanisms of digestion, absorption and movement of food.

8. The demonstration by von Mering of an increased sugar content of the portal blood during absorption of carbohydrates, was an important advance in our knowledge of the mode of absorption of sugar. Similarly, Munk's work on the fat content of the chyle from the thoracic duct and that of Folin, Van Slyke and others (*v.* Cathcart's monograph) (29) on the non-protein nitrogen of the portal and peripheral blood have likewise elucidated the path of absorption of fats and proteins respectively. It is strange that it is only within comparatively recent times that a comparison between the composition of the chyle and of the portal blood has been attempted. This was done first by Hendrix and Sweet (30) for sugar and later, for sugar, by Sataké *et al.* (31) (32) and others, and for amino N by Ishikawa (33). The significance of such a comparison, which has been stressed by Krogh (34), will be discussed later.

London (35) (36) has devised a method, "angiostomische Methodik," by which blood can be drawn off from selected abdominal vessels, at repeated intervals from the same animal. He attaches a special metal cannula to connective tissue in close proximity to the vessel. It is also fixed into the abdominal wall and communicates with the exterior. Special needles can be inserted into the cannula to puncture the vessel and draw off blood. The method has been successfully used by Kotschneff (37) and others (37a). The necessity for deproteinisation of the blood sample before analysis is the great drawback of these methods.

9. Biological tests (38) (33): In these the presence of foreign proteins in the circulating fluids is tested for by the anaphylactic and precipitin reactions. Obviously, the method is only of very limited application.

It will thus be seen that most of the methods discussed leave something to be desired, either on the ground of accuracy or because they have little direct application to the subject. Solution of the problems will very probably only follow the employment of judicious combinations of the known methods, either amongst themselves, or with whatever new devices progress in the physical sciences may render possible.

Absorption may conveniently be discussed in relation to the structures in the gut-wall, *viz.*, the epithelium, the capillaries and lymphatics, the villi, the musculature, and the nerves.

THE EPITHELIUM. It is generally held that only the epithelium of the villi is concerned in absorption. The reasons are purely anatomical but they are very convincing.

Observations on surviving intestine are more likely, for reasons stated, to throw the most direct light on the rôle of the epithelium. Mielke (39), however, from experiments done in Kestner's (Cohnheim's) laboratory has stated that the epithelium does not survive excision. He killed rats immediately after a carbohydrate meal and found that the concentration of sugar in the small intestine was hypertonic. He concluded from this that carbohydrates were normally absorbed in hypertonic solution. When he placed hypotonic sugar solutions in surviving loops of rat's intestine suspended in oxygenated saline at body temperature for half-an-hour, the concentration did not increase (*i.e.*, did not tend to become hypertonic). On this slender basis, supported, moreover, by an inadequate number of experiments, he argued that the passage of material from the lumen of surviving segments was a question of simple diffusion and not of selective absorption. Verzár (3, p. 11) is sceptical of results obtained by the method, because of the great sensitiveness of the epithelial cells, which are seen to become detached soon after excision of the gut. The results of Magee and Southgate (40), which in certain important particulars confirm those of Adam (41), Lurje (42) and Gayda (43) show that, contrary to Verzár's opinion, the epithelial cells are very resistant to unphysiological concentrations of electrolytes. Thus, it was found that the peristaltic movements in isolated intestine were not affected when the inner fluid was as acid as pH 1.7; but, when the epithelium was previously devitalised, movement ceased immediately by lowering the pH. It is probable that the protective mechanism is similar to that demonstrated by L. Hill (44) for the respiratory mucosa, *viz.*, secretion of mucus which is, presumably, the special function of the goblet cells. There is, therefore, good evidence for believing that the epithelium functions in a more or less normal fashion in the isolated gut.

The ideal method of studying the rôle of the epithelium would be to make an isolated preparation of the mucosa alone. This has not been done nor does it appear possible. In isolated preparations the main diffusion path is, of course, directly through the gut-wall, and Macleod, Magee and Reid (45) have found that the rate of disappearance of sugar solutions from the lumen of isolated segments with the blood vessels tied off was the same as from segments in which the cut blood vessels were left untied. There is no direct evidence that might settle the ques-

tion whether intestinal muscle behaves in an active or a passive manner towards solutes passing through it. Ammon (46) found that glucose diffused through living skeletal muscle of mice more slowly than through similar muscle killed by exposure to dry air; but his results merely raise a point that will be discussed later—the possibility of alteration of the physical state of the membrane of muscle by the method of devitalisation.

It appears probable that the serous and muscular coats play a relatively passive rôle during absorption of solutions injected into the peritoneal cavity. Thus, Cohnheim (47) has shown that the absorption of glucose solutions from the peritoneal cavity proceeds in an entirely different manner from that of similar solutions placed in the intestine of intact animals. Starling (48) contended that absorption from serous cavities was largely determined by the osmotic pressure of the plasma proteins. With this opinion, the results of Clark (49), Putnam (50) and of Cori, Cori and Goltz (51) are generally in harmony. Hara (52) concluded that the endothelium played an active rôle but his methods are not above criticism. It is very probable, therefore, that if different concentrations of the same substance or equimolecular solutions of similarly constituted substances, such as sugars, are placed in isolated intestine, any deviations from physical principles, in regard to the rates of passage from the lumen, are to be attributed to the epithelium and not to the other structures in the wall of the intestine.

It is to be noted that the tissues in surviving segments are already in a dying state, so that results partly in agreement with physical laws are to be expected in all experiments; but in some more than in others, in accordance with the viability of the gut and the nature and the concentration of the solute used. Some recent experiments by Purves (53) in Macleod's laboratory illustrate this point very clearly. The rate of passage of sugar to the outer fluid from the lumen of a segment of rabbit intestine perfused with 13.5 per cent glucose remained constant for about 3 hours and then increased very rapidly just about the time when active movements ceased. It is very probable that the increase in the diffusion rate was due to greater permeability of the gut-wall on account of the death of the tissue.

If the epithelium plays a purely passive rôle in absorption or if the "vital" functions it carries out in life are absent in the isolated segments, then the gut-wall in the latter should behave as a dead membrane; that is, the rate and direction of transfer of solutes should depend upon:

a, the permeability of the membrane,

b, the temperature,

c, the diffusibility of the solute and

d, the solute concentration on the two sides of the membrane.

To what extent is this the case?

Permeability of the membrane: Magee and Macleod (54) (55) and Auchinachie, Macleod and Magee (56) found that various sugars and electrolytes passed more rapidly through the walls of segments of gut devitalised by fluoride, cyanide, heat or exposure than through those of surviving segments of equal weight. In all cases the substance in question was absent from the outer saline solution. These workers are of opinion that their results can be explained by alteration of the physical structure of the gut-wall by the killing agent, so that, in place of being of the nature of an emulsoid, it was changed to that of a suspensoid.

Results obtained by Cohnheim (57) on isolated cat's intestine are here of interest. He found that surviving segments transferred water from the inner to the outer fluid of approximately the same osmotic pressure; but, when fluoride was put into the inner fluid, little or no transport occurred. He concluded from this that the fluoride had changed the gut-wall from a living structure capable of selective absorption into a dead membrane which would behave in a purely physical manner to alterations in its environment.

As chemical or physical agents have been extensively used to devitalise segments of intestine in studies on absorption, it may not be out of place here to discuss their action on living tissues in general. Living protoplasmic membranes differ generally from artificial membranes in two particulars—they possess a structure which cannot be described in physico-chemical terms, and they exhibit in greater or lesser degree the phenomena of life. These characteristics are mutually interdependent. If we destroy the power of protoplasm to continue the processes of life, we simultaneously alter its structure and *vice versa*. Moore (58) has put forward the view that membranes of the nature of any that can be constructed artificially do not exist in the living body; but that during life protoplasm reacts in some way towards local variations in its environment. It may be that, in the case of such membranes as the peritoneum, the protoplasm functions in a relatively passive manner, compared with, for example, the epithelium of secreting glands or of the kidney tubules. In the former, the protoplasm probably manifests its vitality chiefly by altering the permeability of the membrane through which, by osmosis and other physical forces, the passage of water and solutes occurs. In the latter, the protoplasm apparently can perform work by selectively taking up materials and transferring them across

the membrane against the osmotic pressure gradient. The passage through the peritoneum, of materials to which it is permeable, would, if these assumptions are correct, be governed largely by the laws of diffusion and osmosis whereas, in the case of selectively-acting membranes, this would not be so and the transport of materials would be accompanied by an increase in metabolism. Brodie and associates (59), (60) found an increase in O_2 consumption and in CO_2 output during absorption of distilled water, salt solutions of varying concentration and Witte's peptone in loops of dog's intestine. They calculated that the increased respiration was due chiefly to the epithelium, on the assumption that, as the muscular coat did not show any significant movements during the experiments, its metabolism remained constant. It is very questionable if this assumption can be taken as correct, although Lovatt Evans (61) has shown that the O_2 usage of plain muscle during rest is reasonably constant. Unless it is correct, the increased respiration might be attributed entirely to the muscular elements. It is very desirable that these experiments be repeated with the metabolism of the muscular coat controlled as completely as possible.

Höber has suggested on the grounds of numerous experiments with dyes, soluble and insoluble in lipoids (62) (63) (64), that substances insoluble in lipoids pass in through the intercellular spaces while those soluble in lipoids pass directly through the epithelium. This conception would relegate a very insignificant rôle to the epithelial cells in the living animal and a predominant one to the intercellular spaces. Solubility in lipoids unassisted by any other factor would, as Moore (58, p. 318) has pointed out, result merely in the accumulation, to saturation point, of the substances in question in the lipoids of the tissues and could not account for the steady stream of fluids through the epithelium that occurs during absorption.

Temperature: Since it was found impossible to devitalise the gut-wall by chemical agents, by heat or by drying, without at the same time fundamentally changing its structure, Auchinachie, Macleod and Magee (56) tried whether, by lowering the temperature of the environment, the vital properties could be held in abeyance. It may be assumed that, under these conditions, the texture of the gut would not be altered. When equal amounts of the same glucose solution were put in two series of surviving and "dead" segments of rabbit intestine kept in Tyrode solution at different temperatures, (0°, 20° and 40°C.), it was found that the rate of diffusion with living segments increased more rapidly between 20° and 40°C. than between 0° and 20°C. whereas, with dead

segments, the increase was similar for each range of temperature. When equal volumes of equimolecular solutions of glucose and xylose were put in two series of loops of gut kept at 0° and 40°C. respectively, xylose diffused out more rapidly than glucose at 0°C., but glucose more rapidly than xylose at 40°C. Furthermore, Reid, in this laboratory, has found that when the experiment was varied by suddenly raising the temperature from 0° to 40°C., after an hour at the lower temperature, the diffusion curves crossed, thus showing a more rapid rate for xylose at 0°C. but for glucose at 40°C.

Diffusibility of the solute: This is the most appropriate place to consider the work of Höber (62) (65) and of his pupil, Katzenellenbogen (67), although their experiments were done almost entirely on loops *in situ*. Höber contended that the rates of absorption of various substances proceeded in accordance with Van't Hoff's theory of solutions and Arrhenius' theory of electrolytic dissociation, but he admitted that not all the phenomena could be explained in this way. On the other hand, Wallace and Cushny (68) obtained results contrary to Höber's theory, in experiments on anesthetised dogs and cats. Irving (69), taking the Ca content of the blood as the index of absorption, found in dogs that the rate for salts of Ca varied as follows: acid acetate > neutral chloride > acid citrate > acid lactate. He suggested that the acidity of the salt was the factor that determined the rate of absorption.

The formulation of theories of absorption based on results obtained by the use of solutions of inorganic salts or of substances that do not find a place in mammalian dietaries is open to criticism. Compared with the amounts of sugar, amino-acids and fats that daily pass through the intestinal wall, the amount absorbed, of even the most important of the inorganic salts, is relatively very small. In absorption experiments the inorganic salts, in most cases, have to be employed in unphysiological concentration in order to make chemical analysis possible, so that an action of an irritant nature or one in opposition to the normal functions of the epithelium would very likely be initiated. This criticism, first made by Starling (48, p. 52) in discussing Höber's experiments, applies also to the recent work of Mond (17), who found that the permeability of the frog's gut for the dye, cyanol, could be varied by wide variations in the concentration of electrolytes in the gut-lumen or in the blood vessels.

It will be clear, then, that a better insight into the mechanism of absorption is to be gained by the employment of solutions of sugars or amino-acids than of any other solute.

Macleod and Magee and their colleagues, as stated above, found that glucose disappeared more rapidly than xylose from equimolecular solutions placed in surviving segments at body temperature. The relative rates of diffusion of the two sugars with "dead" segments were in the reverse order, as would be expected from the smaller size of the pentose molecule. Many other experiments comparing the absorption of various sugars have been done, but only on living animals or in segments of gut with intact circulation. As most of them show results in harmony with those obtained on surviving loops, one may assume that the selective absorption in both cases was attributable to the intestinal epithelium, and not to any other structure in the gut-wall.

According to Nagano (12), the series galactose > glucose > fructose > mannose > xylose > arabinose expresses the relative rates of absorption of these sugars from Vella fistulae, which is in precise agreement with the results of Cori *et al.* (18) (19). Hédon (7) also showed that glucose and galactose were absorbed by rabbits more rapidly than arabinose. The series sucrose > maltose > lactose gives the order for the absorption rates of disaccharides, as found by Röhmann and Nagano (70). Hewitt (4), clearly on insufficient evidence, contended that glucose was absorbed more rapidly than galactose or fructose. Höber (65) found that galactose and glucose were absorbed more rapidly than sucrose or lactose, and contended that this result supported his theory that absorption ran parallel with diffusibility. He was not aware of the facts that the latter are hydrolysed either in the lumen or in the epithelial cells and that relatively little lactase is produced by the mucosa of adult animals (70). The time required for hydrolysis would, presumably, account for the delay.

The solute concentration: Using surviving cat's intestine, Cohnheim (57) demonstrated transport of glucose and NaCl from solutions of various concentrations placed in the lumen, the outer fluid consisting of Ringer's solution, blood serum or a mixture of the two. With the isolated gut of *Holothurians* he found that sea-water was taken up from the lumen and transferred to the outer fluid consisting also of sea-water (71). In these experiments the absorption path was the normal one for this species. These results were in general confirmatory of his other work on loops of intestine *in situ* in living animals (16) (47), or in animals that had just been killed (72). He also found that iodides dissolved in sea-water were completely taken up by the isolated gut of Octopus and transported into the medium of the animal's own blood in which it was suspended (73).

Reid (74) (75) found that when two vessels were separated by a membrane of surviving rabbit's intestine and each filled with serum or Ringer's solution, a transfer of fluid occurred from the epithelial to the serous side. Results showing absorption of water and solids from solutions of various concentrations or from blood serum were obtained by Reid (76) (77), Heidenhain (78), Rabinowitch (79) and others on loops of gut with intact circulation. Hamburger (80) contended that he got similar results by inserting horse serum, or hypertonic and hypotonic solutions of NaCl into the intestine of dead dogs and rabbits. His findings were not quite the same as those of the workers he criticised for, while Reid, Heidenhain and Cohnheim always observed absorption of water and solids, Hamburger found a decrease in volume but an increase in the concentration of solids after injecting serum. Goldschmidt and Dayton (81) were not able to confirm Hamburger's results when they tested the fate of NaCl solutions on the colon of dead dogs.

Hamburger (82) also claimed that results obtained by him with diaphragms prepared from intestine of horses, dead for 17 hours or more, differed only in degree from those got with living intestine. He worked with a specially constructed apparatus and employed horse serum as an outer fluid, which was sometimes in contact with the epithelium and sometimes with the connective tissues. Some experiments indicated a more rapid transport of water, sugar or NaCl from epithelium to connective tissue, and some reversely. The direction of greatest diffusion was at times the same for all three substances, at others it varied. Such results, especially in view of the long time allowed for diffusion, about 15 hours, have no parallel with any obtained with living intestine.

The rate of diffusion of glucose through "dead" intestine varies directly with the concentration of the solution; but, we have found that while this was also the case with surviving gut up to 3M/4 strength, the rate for M/1 solutions fell away instead of increasing. Thus, the optimum concentration for glucose absorption from the isolated surviving intestine is 3M/4 (56). This concentration (13.5 per cent) is much greater than that of an isotonic solution, which was the optimum suggested by Cohnheim (57).

That the rate of absorption of a solute from the alimentary canal should remain constant or approximately so from hour to hour, notwithstanding continual diminution of its concentration, is entirely contrary to physical laws. Cori and his associates (18) (19) (83) have found this to be the case for glucose and for amino-acids. Their results have been in general confirmed for amino-acids (Wilson and Lewis) (84) and

for sugar (Auchinachie *et al.*) (23). Pierce, Osgood and Polansky (20) (21), however, have brought forward results and arguments to show that the rate of absorption for glucose falls off from the time of feeding. In their reply, Cori *er al.* (86) submit new evidence in support of their opinion and point out that Pierce *et al.* did not feed enough sugar to enable absorption to proceed for three hours. Furthermore, the calculations of Pierce *et al.* do not seem very convincing. Cori's opinion may then be taken as substantially correct; but all the published results, including his own, show a slight falling off in the rate as absorption proceeds. It may be that the gastro-intestinal movements have something to do with this. However, when the figures are compared with those for absorption from the peritoneal cavity (51) the contrast is very striking. Compared with the latter, the rates for the intestine are relatively very constant.

It is interesting to note that the concentration of the unabsorbed sugar in Cori's experiments fell from 80 per cent to 14 per cent after 4 hours (19). Further work with the object of testing these results and also of determining the normal concentration of sugar in the gut during digestion of a carbohydrate meal is desirable.

Not all substances, however, are absorbed at a constant rate from the intestine, for the Coris (87) have found that the rate of absorption of lactates varied directly with the amounts fed. It is also noteworthy that the passage of KI from the isolated living gut of rabbits varies directly with the concentration of the salt (56). These results would suggest a mode of uptake for some substances from the lumen which is more in accordance with physical laws than for others such as sugars, amino-acids, NaCl and water.

Summarising all the evidence considered under the above four heads, it will be seen that the majority of the experimental results cannot be explained by known physical laws. The conclusion may therefore be drawn that the phenomena of absorption are due partly to some special property of the epithelial cells.

OTHER FACTORS INFLUENCING ABSORPTION. *Level of intestine.* Auchinachie, Macleod and Magee (56) found, in general, that the rate of disappearance of solutes from the lumen increased analwards, a result to be explained most probably by the decreasing density of the muscular wall in this direction. In loops with intact circulation, on the other hand, Nagano (12), Röhmann and Nagano (70) and Frey (88) showed that the absorption of sugars was greater in the duodeno-jejunum than in the ileum. Ishikawa (33) found the ileum to be more permeable for

foreign proteins than the jejunum; but, as he used the precipitin and anaphylactic tests as an index of absorption, his findings are scarcely relevant. London and Polowzowa (89) found, in polyfistula dogs, that absorption of glucose was greatest in the ileum. Their results were very probably complicated by the movements of the intestines.

Surface tension: Traube (90) has suggested that surface tension phenomena are very important factors in absorption. His views are based chiefly on theoretical considerations and on the work of Overton, Höber, Katzenellenbogen and others. He would explain the absorption of serum and plasma from the gut on the grounds of the lower surface tension at the interface, lipoid-water, than at the interface, protoplasm-water. Török (91) has tested his hypothesis and found that additions of oil and gum arabic had no influence on absorption of NaCl solutions. Similarly, Buglia (92) found that numerous substances, including bile, which he showed had a definite lowering effect on the surface tension of solutions of NaCl and peptone, had no, or only a slight retarding, influence on absorption of such solutions in dogs. Traube (93) quotes work by Brown (94) to support his thesis. Brown formed the opinion that the semipermeability, in regard to inorganic and organic solutes, that he demonstrated for the envelope of the barley seed, was due to the attraction of the molecules for water but was independent of diffusibility, degree of ionisation, surface tension or viscosity of the solutions. His results do not appear to offer much support for Traube's theory, but some more recently published are suggestive in this connection. Thus Langecker (95) has shown that bile acids increased absorption of glucose and drugs in dogs and amphibia, and Lasch (96), that saponin increased calcium absorption from isolated segments. Similar results were obtained for glucose in isolated segments, and confirmed by oral administration to living animals (Lasch and Brügel), (97) and for other substances (Endo *et al.*) (98). It is interesting to note that administration of saponin has been found to increase the amount and fermentative power of the digestive secretions (Petschacher *et al.*) (99); but the stimulation of glucose absorption cannot be accounted for in this way, as it does not require any further digestion.

The evidence in regard to the rôle of surface tension is therefore contradictory. It may be that the effect of saponin is due to stimulation of the epithelium and not to its influence on surface tension.

It was held for many years that fats are absorbed chiefly as soaps. This view is no longer tenable for, as cited by McClendon and Medes (100) and Verzár (3, p. 61) (101), the contents of the small intestine are

almost invariably acid. Soap formation must then be a rare occurrence. Indeed, Jarisch (102) has brought forward evidence which makes it extremely improbable that soaps can exist anywhere in the body unless in very small concentration. Verzár and Kuthy (103) (104) (105) have demonstrated in numerous experiments that the bile acids can combine with fatty acids to form double compounds, stable at reactions as acid as pH 6.18, and Verzár, therefore, believes that fats are absorbed in this form into the epithelial cells (3) (101). The compounds are broken down there by a reaction more acid than pH 6.18 or by the presence of relatively large amounts of alkali salts in which they are also unstable. Determinations of the pH of the interior of intestinal epithelial cells by a method such as that of Chambers (106) (107) (108) would probably elucidate this point.

Mellanby (109), in a recent preliminary note, reported experiments favouring the view that neutral fats are absorbed as such but, so far, he has not substantiated these findings. Verzár's scheme of fat absorption would, therefore, appear the most probable, although it is desirable that it be subjected to further test by other workers.

It is of interest here that, while the balance of nitrogen and carbohydrates in dogs with seven-eighths of the small intestine resected was found to be positive, it was negative in the case of fat (London and Dmitriew, 110).

Electrical phenomena: No experiments have been done to determine whether a difference in potential exists between the two sides of the gut-wall, but Mond (111) has done so for the frog's stomach, in which was placed a physiological saline solution identical with that perfusing its vessels. Under these conditions the mucosal side was found to be negative to the blood side to the extent of 50 m.v. or more. The difference could be easily altered on the blood side by altering the cation content of the perfusate, but toxic substances such as cyanides were necessary to alter it on the side of the mucosa. It is very probable that similar potential differences occur in the intestine. Such differences might account for the findings of Weise (112) or Girard (113), who showed differences in the rate of absorption of the separate ions derived from salts placed in loops of intestine of living animals. But while one showed that the cation (Mg^{++}) was absorbed more rapidly than the anion (SO_4''), the other found that the anion (NO_3') disappeared at a greater speed than the cation (Mg^{++}). The data are, therefore, too meagre and too discordant to attach any special significance to them in regard to absorption. It may be that the differences of potential observed

are simply an expression of cell activity, such as is found throughout the body.

Chiefly on theoretical grounds, Höber (114) has suggested that electrical phenomena are capable of playing an important rôle in absorption. He suggests that these are of the nature of cataphoresis, but the only experiments he cites in proof of this view relate to blood cells and not to the intestine.

There is some evidence that the permeability of the epithelium is affected by the electrolyte content of the fluid bathing it. In experimental hyperglycemia, sugar is not usually found in the gut lumen, but if the NaCl content of the blood is raised sugar passes into it (Fischer and Moore) (115). Lasch (116) (97) has also shown that the absorption of calcium from surviving loops is greatly increased by raising the NaCl content of the outer fluid.

THE CAPILLARIES AND LYMPHATICS. There are very few experiments supporting the finding of Heidenhain (117) of an increased flow of lymph from the thoracic duct during absorption. Eckstein (118) found increases in lymph flow after injecting fat emulsion into the duodenum, but they were too small to account for the transport of sugar and amino-acids to any significant extent. In support of this statement it has been shown that absorption from a loop of intestine was not appreciably affected by ligaturing all the lacteals (Reid) (119).

It is probable that the sugar and amino-acid contents of the thoracic duct lymph are, as Katsura (120) has suggested (for sugar), a secondary and necessary result of the concentration of these in the hepatic and mesenteric blood vessels. This is the more likely since the sugar content in the former is higher than that in the portal vein during fasting —an apparent sequence of liver glycogenolysis.

The evidence, on the whole, is against any significant synthesis of protein from amino-acids and peptone in the gut or capillary walls (29). More recently, an increase in the polypeptide content of the portal blood during absorption of amino-acids has been reported (Kotschneff) (120a); but synthesis in the blood itself may have occurred. The synthesis was only partial, and complete reconstruction of protein is not suggested. The weight of evidence is also against any considerable change in the constitution of the absorbed glucose. Transformation of α- and β-glucose into the γ form in the intestine was reported by Hewitt and Pryde (121), and Winter and Smith (122) believed they found γ glucose in the circulating blood. These results have not been confirmed for the former workers (Stiven and Reid, 123) or for the latter

(Eadie) (124). Furthermore, Charit (124a) was not able to find an increase in the glycogen of the portal blood during absorption of carbohydrates.

White and Rabinovitch (10), using dogs with Thiry-Vella fistulae, found agreement between the reducing and rotatory values of glucose solutions remaining unabsorbed in the loops and, in Macleod's laboratory, Purves (53) has found similar agreement for the glucose that passed out from aseptically set-up surviving segments. It has not, therefore, been demonstrated that glucose undergoes any transformation in the lumen or in passing through the wall of the intestine.

When sucrose passes through the isolated rabbit's gut it is completely inverted (56), but it is not possible to say definitely whether inversion occurs in the lumen or inside the epithelial cells.

Assuming then that amino-acids, polypeptides and glucose pass unaltered through the epithelium, the mode of uptake of these and of the water and salts by the blood has to be considered. Cohnheim (57) inferred from his experiments that solutions of any concentration tended to become isotonic in the intestinal lumen by *a*, more rapid absorption of water than of solid in the case of hypotonic solutions and *vice versa* for hypertonic solutions; *b*, dilution of hypertonic solutions owing to passage from the blood of water, containing only traces of NaCl. Many results bearing on this point have been obtained (78) (76) (88) (19) (125) (126), but the evidence is very confusing. The general tendency, however, seems to indicate a two-way permeability of the epithelium and capillary wall for water, but only from the lumen outwards for NaCl.

Cobet (127) (8) obtained results showing increases in the N and alkali contents of the intestine of dogs after inserting concentrated solutions of $MgSO_4$ or Na_2SO_4 along with 0.6 per cent. NaCl. He attributed these findings to secretion of intestinal juice. This is a complication that does not appear to have been sufficiently considered by most of the earlier workers who based their opinions on changes observed in the volumes of solutions originally placed in the lumen. Rabinovitch (79) has corrected for this source of error in his experiments. It is probable that, what many regarded as a transudate of water from the blood in response to hypertonic solutions in the lumen, was not so much an expression of a two-way permeability of the absorbing epithelium for water as the result of stimulation of the secreting glands by contact with the solutions. Results pointing to an active secretion have been obtained by others (70) (88).

On the other hand Krogh (34), on theoretical grounds, is of opinion that the lumen contents are in hypertonic solution during absorption, a view which Meikle's (39) results support. The suggested optimal concentration of 13.5 per cent (56) and the constant rate of absorption of concentrated solutions of sugar and amino-acids (18) (19) (83) (84) would also seem to strengthen his contention.

There can be no doubt but that the individual crystalloids are presented to the capillaries in higher concentration than they exist in the blood, so that their entry therein might be explained by diffusion. This concentration gradient and the attraction of the plasma proteins for water would be opposed by the blood pressure in the capillaries. The latter would seem to be in the neighbourhood of 15 cm. of water, (*i.e.*, about four times less than the osmotic pressure of the plasma colloids) as calculated from Carrier and Rehberg's data (34, p. 220). This estimate is probably not far from the truth, for Landis (128) has obtained values of from 10 to 15 cm. for the capillaries of frog's mesentery, by a modification of Chambers' micro-injection technique. The uptake of all the absorbed water and crystalloids by physical means is therefore conceivable, but this cannot be taken as proved.

The area of the capillary surface that abuts directly on the under surface of the villus epithelium is about one-third the surface area of the latter, and the total area of the capillary surface approximately the same as that of the villus surface (Krogh, 34, p. 14). We may assume that the entire epithelium of the villus, and not only those cells lying immediately above and adjacent to the capillaries, participate to an equal degree in the absorptive process. According to Krogh's measurements about one-third the absorbed fluids would pass directly into the capillaries and two-thirds into the pericapillary spaces. It is questionable whether these are anatomically continuous with the lymphatic vessels of the villi, although Krogh evidently regards this to be so. The structure generally given of the central lacteal is that of a closed vessel, so that, to enter either of the circulating fluids, absorbed material would have to pass through the walls of the central lacteal or of the capillaries. The determining factors in the competition are the relative permeabilities of the respective walls and the rates of flow of the fluids. While the permeability is very probably greater in the case of the lacteal, the relatively slow movement of the chyle would soon lead to equilibrium with the pericapillary spaces. This would be impossible in regard to the blood on account of its rapid flow, so that the diffusion gradient would be sharper towards the capillaries. The path to the blood would

then seem the easier. If Krogh's (p. 245) suggestion is true, that the capillary walls are permeable in both directions to water and crystalloids, the probability of uptake by the blood and lymph in accordance with physical laws is strengthened.

While Hendrix and Sweet (30) and Sataké *et al* (31) (32) have shown that the sugar content of thoracic duct chyle and portal whole blood follow approximately the same course, Katsura (120) found that portal serum (which, and not whole blood, he maintains is to be compared with chyle) contains more sugar than the chyle during absorption of carbohydrates. Katsura, having shown equal amounts of sugar in hepatic vein serum and thoracic duct chyle, concluded that the composition of the latter was an expression of the equilibrium existing between the intestinal and hepatic capillaries and the lacteal chyle. Comparisons of the mesenteric lymph and blood are obviously desirable.

Having entered the central lacteal, the contents are evidently moved onward by the contractions of the villi and of the muscular coats, and probably by rhythmical contraction of the lacteals themselves, which has been demonstrated by Florey (129) (130). A further exchange with the blood very probably occurs at the venous nests of Mall in the submucosa (34, p. 15).

It will be convenient here to refer to the absorption of fats.

The fats: The passage of fats from the epithelium into the circulating fluids requires separate treatment because, unlike the other absorbed materials, they can be detected histologically. It is proposed only to touch lightly on this subject because it has been reviewed recently by Bloor (131), Leathes and Raper (132) and Verzár (3).

Our ideas as to the channel of fat transport are still dominated by Munk's classical experiments on a human patient with a lymphatic fistula. Only 60 per cent. of the fat absorbed could be detected in the lymph and the immediate post-absorption history of the remaining 40 per cent. is still obscure.

Advancement in this field has clearly been hampered by the lack of a suitable method for estimating the fat content of small amounts of blood. Bloor and his colleagues (133) (134) have developed a procedure requiring only 5 cc. of blood. The method is a nephelometric one and for this reason is open to criticism. Recently Nedswedski (135) found, by Bloor's (134) method, more fat during absorption in the portal than in the femoral blood, and thus confirmed D'Erico's earlier findings. Nedswedski obtained the blood for his analyses from the femoral artery and portal veins of unanesthetised "angiostomierten" dogs (35) (36).

Regarding the mode of transfer of the resynthesised fat from the epithelium into the central lacteal of the villi, the position is still obscure. The chief theories that have been adduced are:

1. The expulsion theory of Heidenhain which ascribes contractile powers to the epithelium,

2. The phagocytic theory of Schafer, according to which the fat globules gain entrance in some way into the leucocytes found in abundance in the mucosa, and are carried by the latter to the central lacteal where they are set free by disintegration of the cells.

3. The hydrolysis-resynthesis theory of Loevenhart (136). This theory is based upon the reversible action of lipase on fats, so that, depending upon the masses of the reacting substances, hydrolysis or synthesis would take place. Loevenhart believed that these processes can occur at cell boundaries throughout the body, because he demonstrated the presence of lipase in the body fluids and in many organs where fat is usually deposited. His hypothesis has been tested by Bradley (137), who did not find any broad correlation between the fat and lipase content of tissues. These results do not, however, negative the hypothesis, since, as Bayliss (138) has pointed out, the amount of enzyme does not affect the equilibrium position but merely the rate of the reaction and, as fat is usually laid down very slowly in deposits; therefore, the rate of synthesis cannot be a matter of much importance. Furthermore, the lipase of the blood would allow of hydrolysis or synthesis at the cell boundaries of tissues which do not contain any lipase at all. It may also be remarked here that, on theoretical grounds, the lipase content of tissues should depend largely on their ability to catabolise fat as well as on their ability to store it.

It will be clear from this discussion that the question of the transfer of fat from the epithelial cells to the lymph or blood is essentially the same as that of fat transport and deposition in the body in general. It may even transpire that the history of fat in the body, from the time it enters the lumen of the intestine until its mobilisation for catabolism, depends upon the same fundamental process, with modifications according to the demands of individual tissues and of the body as a whole. And, just as the bile salts promote uptake of the fatty acids from the gut, so there may exist in the body tissues or fluids small quantities of bile salts which would, in a similar way, assist transfer of fat from the capillaries to the cells. It is conceivable that bile salts are set free by the reticulo-endothelial system from phagocytosed erythrocytes at the same time as bile pigment.

THE VILLI. Brücke's conception of the importance of the pumping action of the villi in absorption received little more than speculative support until recently, when King and Arnold (139) and Verzár and Kokas (25) studied their movements by improved technique. The muscle of the villi is in close anatomical connection with the *muscularis mucosae*. Strong stimulation of the splanchnics causes ridging and pitting of the mucosa with vigorous villus contractions; but there is no evidence that this relationship is of any importance in absorption. The contractions involve no alteration of the shape of the villi, which partially disappear while the normal rhythmical movements are in progress; so that distortion or obliteration of the capillaries to any great extent is improbable. These authors maintain that the chief result of the contraction is to expel fluid from the lacteal. Probably transfer of water and crystalloids into the capillary blood would also be furthered by diminishing the filtration pressure of the latter. Comparisons of the pressure in the capillaries and lacteals of the villi are desirable.

Verzár and Kokas calculate that 360 cc. of lymph would be expressed per hour by the villi of the dog's small intestine. If an increase of this amount occurred in the chyle from the thoracic duct during absorption, it could easily be detected. As an increase approaching this has not been clearly demonstrated, a further exchange with the blood vessels, probably in the venous nests of Mall, may be postulated.

Hypertonic solutions were found to cause shrinkage of the villi and hypotonic solutions, swelling. The hypertonicity of the lumen contents in absorption would, therefore, induce shrinkage of the villi. Heidenhain's opinion (34, p. 247) that the intercapillary spaces are almost entirely obliterated during absorption would then seem to be not far from the truth; so that the absorbed materials would have a more direct passage into the capillaries through the compressed tissues of the villi. Although it is very probable that the villus movements have an important function to perform in mammals they are evidently not absolutely essential; for absorption takes place in the isolated gut of *Holothurians* which has no villi (71).

THE MUSCULATURE. *Filtration:* Filtration was believed by Lieberkühn and by others since his time to be the predominant factor in absorption. The theory demands a higher intra-intestinal hydrostatic pressure than that in the circulating fluids in the villi. Obviously, such conditions would lead to obliteration of the capillaries and lacteals and, if filtration occurred at all, the fluids expressed would pass into the peritoneal cavity and only very slightly into the blood or lymph. Work

done by older observers, purporting to show increased absorption as the result of increased hydrostatic pressure, was vitiated by the fact that the surface area of the gut was greatly increased by the pressure. Hamburger (80) took precautions by means of a special device to avoid this in anesthetised animals, and found that absorption was accelerated by increasing the intra-intestinal pressure. He repeated these experiments but raised the intra-intestinal pressure indirectly, by increasing the intra-abdominal pressure (140). Again, he found that the rate of absorption varied directly with the intra-intestinal pressure and that absorption ceased when the pressure was reduced to or below zero. He therefore concluded that the intestinal and respiratory movements were, with molecular inhibition, the determining factors in absorption.

While these experiments are no proof that filtration plays any part in absorption, they suggest that the intra-intestinal pressure is a factor of some importance. It can only be a minor one because, in conditions such as cholera involving increased peristaltic contractions and, presumably, increased intra-intestinal pressure, diarrhea and not increased absorption occurs. Furthermore, absorption from the stomach is normally negligible although, in it, the pressure is probably as high as in the intestines.

Magee and Southgate (40) found that rhythmical intestinal movements did not occasion any significant increase in pressure in segments about 8 to 10 cm. long. It is very probable, however, that while powerless to provoke a general rise of pressure throughout the intestine, they produce local changes in pressure especially in those animals in which they are truly segmenting in type as in man, the cat and cavy. No measurements of this pressure have evidently been made.

Magee and Southgate (40) found "diastolic" pressures as high as 20 cm. of water in surviving segments of cat's intestine during optimal peristaltic contractions. At the height of contraction the pressure rose several centimeters above this, so that, if such pressures occurred in the gut of living animals, the capillaries would certainly be obliterated. That this occurs is shown by the frequently observed fact that the gut in the living animal becomes pale at the spots where vigorous peristalsis is in progress.

Experiments by Landis (141) are here of interest. He showed that lack of O_2, induced by experimental stasis, rapidly increased the permeability of the capillary wall in the frog's mesentery, so that at first a protein-free, and later a protein-containing, filtrate passed out from the blood. The rate of transudation varied directly with the difference

between the pressure of the blood and the osmotic pressure of its proteins. On readmitting oxygenated blood the normal semipermeability of the wall was restored. It would be of great interest to know whether the local and temporary anemia, induced by normal vigorous contraction of the musculature, temporarily increases the permeability of the capillaries. If this were shown to be so, passage of solutes into the blood would be accelerated immediately following peristaltic or vigorous segmenting contractions.

It is very probable that the threshold pressure for the initiation of peristalsis in man is higher than that found in laboratory animals. In the cat's duodenum it is as high as 12 cm. of water (40); so that even before peristalsis begins in man, the intra-intestinal pressure probably exceeds that in the capillaries.

On the whole, then, it appears probable that the intra-intestinal pressures developed on account of the respiratory and intestinal movements promote absorption by hurrying onwards the flow of blood and lymph, by counteracting the capillary blood pressure and, perhaps also, by temporarily increasing the permeability of the capillary walls.

THE NERVES. Reid (143) showed that the effects of section and stimulation of the mesenteric nerves on the rates of absorption of peptone and water were probably attributable to the vasomotor changes induced. Borchardt's (144) results support Reid's. The existence of specific absorptive nerves has not, therefore, been shown.

INTESTINAL FUNCTION IN RELATION TO THE DUCTLESS GLANDS. *The adrenals:* It has been abundantly shown that circulatory and ulcerative lesions of the gastro-intestinal tract are common *post-mortem* findings in adrenalectomised animals (145) (146) (147) (148) (148a). Magee *et al* (149) have not only confirmed these results in rabbits, but have observed hemorrhages and erosions in the region of the pyloric sphincter in unilaterally adrenalectomised cavies.

Conversely, McCarrison (150) and Magee *et al* (149) have observed, not only lesions in the gastro-intestinal mucosa, but also circulatory and degenerative changes in the adrenal cortex of animals fed on inadequate diets. The latter have ascribed these conditions to over-stimulation of the glands which, in the opinion of Cramer (151), could be brought about by absorption of toxins resulting from intestinal putrefaction of proteins. Cramer's conception is supported by the observations of Dale (152), Crivellari (153) and Scott (154), who showed that adrenalectomised animals are abnormally sensitive to histamine. These results suggest that some relationship exists between the functions of

the cortex and absorption of toxic substances from the alimentary canal. This is all the more likely since it has been shown that the lethal dose of extract of the intestinal contents, got from artificially obstructed loops, is much lower for adrenalectomised than for normal animals, after injection (Cutting) (155).

Results have also been obtained indicating that histamine, after introduction into the intestine of normal animals, is rendered pharmacologically inert in its passage through the intestinal wall (Koessler and Hanke) (156). If, however, devitalising agents, such as chloroform, were inserted simultaneously into the intestine, definite falls in blood pressure were registered (Mammoser and Boyd) (157). Evidence has also been produced indicating that the factor chiefly responsible for the toxemia of intestinal obstruction is the devitalisation of the mucosa and not the production of toxins (Wagensteen) (158), (Cooper) (159). Indeed, extracts of the intestinal contents from normal dogs were found to be just as toxic on injection into normal dogs as similar extracts got from dogs suffering from experimental intestinal obstruction and its accompanying toxemia.

Weighing up all this evidence, it would seem that the intestinal mucosa is normally endowed with important detoxicating powers and that the adrenals are related in some way to this or other functions of the epithelium. Thus, there is evidence to suggest that they are related in some way to absorption, for the Coris (160) have shown that the rate of absorption of glucose is greater in normal than in adrenalectomised rats. Furthermore, McCallum and Magee (161) have brought forward evidence suggesting a close relationship between the cortex and the motor functions of the small intestine.

The thyroid: Changes in the structure of the thyroid in consequence of inadequate diets have been reported by Sharpey-Schafer (162), Harris and Smith (163) and Murray (164). Orr and Leitch (165) are of the opinion that the thyroid is definitely related to intestinal function. They quote as evidence the occurrence of constipation in myxedema, and of diarrhea due to thyroid administration.

The parathyroid: There can be no doubt but that the increase in the Ca and P of the blood after administration of the hormone is due to their liberation from the bones and not to increased absorption. Recently, Taylor and Fine (166) have shown that the excretion of calcium by the small intestine of parathyroidectomised cats is much greater than in normal cats; so that the parathyroid may have something to do with the permeability of the epithelium for calcium.

The pituitary: Evans and Long (167) have induced gigantism in rats by feeding preparations of the anterior lobe: a result which has been confirmed in rats and dogs (168). Experiments, suggesting that this lobe is not anabolic or metabolism-sparing in function, have been reported (Lee *et al.*) (169), so that stimulation of absorption by it is probable.

Pregnancy and lactation. In pregnancy the progressively increasing formation of new tissue is not the result of a slowing of the rate of metabolism; for Magnus-Levy and others (170) have demonstrated a progressively increasing rate of consumption of oxygen. Furthermore, Magee (171) found, in the pregnant goat, that the metabolism per kilo body-weight also progressively rose. This finding has been confirmed on humans by several observers (172) (173) (174); so that progressively increasing amounts of food begin to be absorbed soon after conception.

In lactation the mother has to absorb sufficient food, not only for her own wants, but also for transformation into milk for her offspring, so that again the rate of absorption must be greatly accelerated. Not only is the rate of metabolism not subnormal in lactation, but values above those in the non-pregnant and non-lactating state have been reported (175) (171). In dairy animals, in which pregnancy and lactation run concurrently for considerable periods, the absorptive functions have to meet the twofold demand. There is no evidence to indicate clearly how this stimulation of absorption in pregnancy or lactation is brought about.

INTESTINAL FUNCTION IN RELATION TO THE FOOD INGESTED. Under this heading the influence of the composition of the diet and of fasting will be discussed.

Deficient diets. Mottram, Cramer and Drew (176) found that absence of vitamin B and to a large extent of vitamins A and D altered the histological picture of the intestinal epithelium during fat absorption. The effects were immediate, having been observed in animals during the absorption of a single meal deficient in the vitamins. After a normal feed, two streams of very fine fat globules were observed on either side of the nucleus. After a feed deficient in vitamin B, large globules of fat were seen between the free border and the nucleus, squeezing the latter down towards the basement membrane. Cramer and Ludford (177) further showed that the Golgi apparatus swells up and enlarges during absorption of fat, and that the particles of resynthesised fat occur in the meshes of the network. These changes were almost entirely absent in the epithelium of rats and mice suffering from lack of vitamins

A and D or B. Never (15) concluded from experiments on dogs with Pavlov pouches and intestinal fistulae that the absorptive, and not the digestive functions, of the gut were impaired in avitaminosis. Bergeim (178) also contended, from preliminary experiments, that vitamin-free diets did not significantly damage the digestive functions. More direct proof that vitamin B has a stimulating action on absorption has been brought forward by Kokas and Gal (22), who showed by Cori's technique that additions of yeast extract to solutions of glucose and peptone markedly accelerated their absorption.

It is generally believed that the beneficial effect of vitamin D on bone formation is due to stimulation of Ca and P absorption. Bergeim (179), however, is of opinion that the vitamin acts by diminishing the abnormal excretion of Ca and P that takes place into the large intestine of animals suffering from rickets. It is of interest to note that Walsh and Ivy (11) recovered, from normally fed polyfistula dogs, greater amounts of Ca in successive washings from the large than from the small intestine.

Irradiation by ultra-violet light has been shown by Orr and his colleagues (179a, b,) and others to increase retention of Ca by diminishing fecal excretion. The subject has been reviewed by Laurens (180). It would appear that ultra-violet light in some way induces the irradiated animal to elaborate vitamin D in its own tissues (180). But doubt exists as to whether the effects of this vitamin or ultra-violet light are produced primarily on the intestinal functions (absorptive or excretory) or on metabolism, because practically all the relevant data have been obtained by comparing the amounts of Ca and P excreted with those ingested over a period.

There are very many experiments demonstrating the production of organic lesions, varying degrees of atrophy and disordered motility in the gastro-intestinal tract as a result of feeding diets poor in vitamins or inorganic elements (149) (150) (181) (182) (183). The question arises as to whether these structural and functional changes are due to the local action of the deficient food in the gut lumen or, less directly, to change in the composition of the blood. If they are due to local action only, then the functions of the epithelium and of the musculature would very probably be immediately affected by changes in the composition of the lumen contents. We have seen that this is the case with the epithelium in regard to some of the vitamins. As Babkin and Woskresenski (9, p. 852) have shown in decerebrate cats that the peristaltic movements are stimulated immediately by soaps and the segmenting

movements by acids and alkalies inside the lumen, it may be that specific stimulants for the musculature, as well as for the epithelium, normally reside in the intestine. Although more evidence is needed on this point, it is conceivable that when certain (deficient) diets are ingested, adequate stimuli for motility would be wanting in the intestine or, alternatively, toxins having a stimulating or inhibitory action might be formed. Experiments designed to determine the influence of toxins derived from intestinal contents on the movements of surviving gut, when placed in the lumen, would throw light on this question. It is interesting here to note that chemical poisons (fluorides) act at once when tested in this way (40), the movements so initiated being apparently directed towards the expulsion of the irritant. The response shown in these experiments was too rapid to be other than nervous, so that a local reflex may be postulated. It is not difficult, therefore, to imagine how a continuance of excessive stimulation or inhibition from the lumen would give rise to disorganisation of movements. This has been shown, for peristalsis (184) (185) (186) and for rhythmical movements (187), in animals fed on diets deficient in vitamins or minerals. It is, however, also possible that all the above-mentioned changes are to be ascribed to impoverishment of the blood (or to toxemia) which would lead to faulty nutrition of the gut-wall in common with the rest of the body. Weighing up the evidence bearing on these points, it would seem that the pathological effects of deficient diets are due, partly to local and partly to systemic action.

The effects of fasting on intestinal function, while not decisive for discriminating between these alternative modes of action are, nevertheless, of some importance.

Thus, the Coris have found that glucose and fructose are absorbed more slowly by rats after a 48-hour than after a 24-hour fast (160) (188): a result which has been confirmed in this laboratory for glucose. Further, Morgulis (183, p. 197) showed that the absorption of fats by trouts fell by 93 per cent after a fast of two weeks; a finding that might be attributed to impaired digestion or absorption or both. It is probable that absorption was principally affected, as our results with glucose testify.

Fasts of 24 hours or more have been shown to induce progressive degeneration of the mucosa in white mice (Sun) (189), and changes of a similar nature in consequence of undernutrition are quoted by Jackson (182). The effects of re-feeding in Sun's experiments were marked. They were a relatively rapid restoration of structural form. Although

it is very desirable that these experiments should be repeated, they are nevertheless very suggestive that the epithelium is the first to suffer in undernutrition. This conception would involve some degree of dependence of the epithelium on the contents of the lumen for its nutrition; and the occurrence of degeneration of the gastro-intestinal mucosa in animals fed on deficient diets suggests the application of a similar *modus operandi* to conditions of malnutrition.

Effects of various dietary constituents. There is evidence that the absorption of some substances is affected by the presence of others in the intestine. Thus Cori (190) found that when mixtures of glucose and galactose were fed together, the total amount of sugar absorbed was less than that when either sugar was fed alone. Glucose disappeared more rapidly than galactose; a result the reverse of that obtained by feeding the sugars separately (19). Similarly, Cori (83) showed that, when molecularly proportional mixtures of glucose and glycine were fed, the total absorption of glycine and especially of glucose was diminished, although the separate amounts taken up were proportional to the molecular weights.

In balance experiments, Kelly (192) found that the ingestion of small amounts of KI increased the retention of P and N in growing animals. Similarly, Richards, Godden and Husband (193) found that K fed in excess diminished the retention of Ca, P and N, and Magee and Harvey (194) that additions of Ca to the diet stimulated retention, not only of Ca, but also of P and N. These experiments, however, do not tell us whether the effects were produced on metabolism or on absorption. For this objective more direct experiments like those of Kokas and Gal (22) are required.

Bergeim (195) found an apparent stimulation of Ca absorption by additions of carbohydrates and especially of lactose to the diet, an effect which he attributed to the formation of lactic acid by bacterial fermentation.

Hart, Steenbock *et al.* (27) found that pure compounds of Fe would not cure the progressive anemia of white rats brought on by diets of cow's milk; but that the ash or acid extracts of lettuce, maize or liver, or liver alone, would do so. Finally, traces of Cu were found to be as effective as any of these. These experiments, however, do not tell us whether the Cu exerts its effects on the blood-forming tissues or on the intestine.

It is also of some interest that very small amounts of Mn or iodine in the saline environment were found to stimulate the rhythmical contractions of intestinal muscle (196).

It is impossible at present to draw any final conclusion as to intestinal function from the experiments quoted in this section. They do, however, indicate that it can be influenced markedly by the composition of the diet or by the balance of the constituents; so that the nutrition of the entire organism may be affected. This is a point that has been emphasised by Orr (197). Further work designed to determine the oligodynamic effect of inorganic elements found only in minute amounts in the animal body is desirable, in order to localise their primary effects.

BIBLIOGRAPHY

(41) Adam. Zeitschr. f. Kinderheilk., 1924, xxxviii, 378, 386; in Ber. u. Phys. u. Pharm., 1925, xxx, 275.

(46) Ammon. Biochem. Zeitschr., 1928, cxcvi, 441.

(23) Auchinachie, Macleod and Magee. Unpublished results.

(56) Auchinachie, Macleod and Magee. Journ. Physiol. (press).

(9) Babkin. Die äussere Sekretion der Verdauungsdrüsen. 1928, 760.

(145) Banting and Gairns. Amer. Journ. Physiol., 1926, lxxvii, 100.

(138) Bayliss. Monographs on Biochemistry. Nature of enzyme action. 1919, 55.

(26) Bergeim. Journ. Biol. Chem., 1926, lxx, 29.

(28) Bergeim. Journ. Biol. Chem., 1926, lxx, 47.

(178) Bergeim. Proc. Soc. Exp. Biol. Med., 1928, xxv, 457.

(179) Bergeim. Journ. Biol. Chem., 1926, lxx, 51.

(195) Bergeim. Journ. Biol. Chem., 1926, lxx, 35.

(131) Bloor. Physiol. Reviews, 1922, ii, 92.

(133) Bloor. Journ. Biol. Chem., 1914, xvii, xxxvii.

(134) Bloor, Pelkam and Allen. Journ. Biol. Chem., 1922, lii, 191.

(144) Borchardt. Pflüger's Arch., 1928, ccxix, 213.

(137) Bradley. Journ. Biol. Chem., 1912, xiii, 407.

(59) Brodie and Vogt. Journ. Physiol., 1910, xl, 135.

(60) Brodie, Cullis and Halliburton. Journ. Physiol., 1910, xl, 173.

(94) Brown. Proc. Roy. Soc., 1909, lxxxi, 82.

(92) Buglia. Biochem. Zeitschr., 1909, xxii, 1.

(29) Cathcart. Monographs on Biochemistry. Physiology of protein metabolism, 1921, 17.

(106) Chambers, R. Anat. Rec., 1922–23, xxiv, 1.

(107) Chambers and Reznikoff. Journ. Gen. Physiol., 1925–28, viii, 369.

(108) Chambers. Bull. Nat. Res. Coun., No. 69, Washington, D. C., 1929, 37.

(124a) Charit. Pflüger's Arch., 1926, ccxiv, 327.

(49) Clark, A. J. Journ. Pharm. Exper. Therap., 1921, xvi, 415.

(8) Cobet. Biochem. Zeitschr., 1920, cxiv, 33.

(127) Cobet. Pflüger's Arch., 1913, cl, 325.

(16) Cohnheim. Zeitschr. f. Biol., 1898, xxxvi, 129.

(47) Cohnheim. Ibid., 1899, xxxvii, 443.

(57) Cohnheim. Ibid., 1899, xxviii, 419.

(71) Cohnheim. Zeitschr. f. Physiol. Chem., 1902, xxxiii, 9.

(72) Cohnheim. Zeitschr. f. Biol., 1900, xxxix, 167.
(73) Cohnheim. Zeitschr. f. Physiol. Chem., 1902, xxxv, 416.
(159) Cooper. Arch. Surg., 1928, xvii, 918.
(18) Cori. Proc. Soc. Exper. Biol. and Med., 1925, xxii, 495.
(19) Cori. Journ. Biol. Chem., 1925, lxvi, 691.
(83) Cori. Proc. Soc. Exper. Biol. and Med., 1926, xxiv, 125.
(188) Cori. Journ. Biol. Chem., 1927, lxxiii, 555.
(190) Cori. Proc. Soc. Exper. Biol. and Med., 1926, xxiii, 290.
(87) Cori and Cori. Journ. Biol. Chem., 1928, lxxxi, 389.
(160) Cori and Cori. Journ. Biol. Chem., 1927, lxxiv, 473.
(86) Cori, Cori and Goltz. Proc. Soc. Exper. Biol. and Med., 1929, xxvi, 433.
(51) Cori and Goltz. Proc. Soc. Exper. Biol. and Med., 1925, xxiii, 122.
(151) Cramer. Brit. Journ. Exper. Path., 1926, vii, 95.
(177) Cramer and Ludford. Journ. Physiol., 1925, lx, 342.
(153) Crivellari. Amer. Journ. Physiol., 1927, lxxxi, 414.
(155) Cutting. Arch. Surg., 1929, xix, 272.
(152) Dale. Brit. Journ. Exper. Path., 1920, i, 112.
(124) Eadie. Brit. Med. Journ., 1923, ii, 60.
(118) Eckstein. Journ. Biol. Chem., 1924, lxii, 737.
(5) Edkins and Murray. Journ. Physiol., 1925, lix, 271.
(6) Edkins and Murray. Journ Physiol., 1926, lxii, 13.
(98) Endo, Mizoguchi and Naito. In Brit. Med. Journ. epitome, 10/7/29, no. 53.
(167) Evans and Long. Anat. Rec., 1921, xxi, 62.
(115) Fischer and Moore. Amer. Journ. Physiol., 1907, xix, 314.
(129) Florey. Journ. Physiol., 1927, lxii, 267.
(130) Florey. Journ. Physiol., 1927, lxiii, 1.
(88) Frey. Biochem. Zeitschr., 1909, xix, 509.
(43) Gayda. Pflüger's Arch., 1913, cli, 407.
(113) Girard. Skand. Arch. f. Physiol., 1926, xlix, 134.
(1) Goldschmidt. Physiol. Reviews, 1921, i, 421.
(81) Goldschmidt and Dayton. Amer. Journ. Physiol., 1919, xlviii, 419.
(125) Goldschmidt and Dayton. Ibid., 1919, xlviii, 433.
(126) Goldschmidt and Dayton. Ibid., 1919, xlviii, 440.
(80) Hamburger. Arch. f. Anat. u. Physiol., (Physiol. Abt.) 1896, 428.
(82) Hamburger. Biochem. Zeitschr., 1908, xi, 442.
(140) Hamburger. Zentralb. f. Physiol., 1896, ix, 647.
(52) Hara. Biochem. Zeitschr., 1921, cxxvi, 281.
(163) Harris and Smith. Amer. Journ. Physiol., 1928, lxxxiv, 599.
(27) Hart, Steenbock, Waddell and Elvehjem. Journ. Biol. Chem., 1928, lxxvii, 797.
(7) Hédon. Arch. internat. de pharmacodyn., 1900, vii, 163.
(78) Heidenhain. Pflüger's Arch., 1894, lvi, 579.
(117) Heidenhain. cit. Krogh, book, 1924, 247.
(30) Hendrix and Sweet. Journ. Biol. Chem., 1917, xxxii, 299.
(38) Hettwer and Kritz-Hettwer. Amer. Journ. Physiol., 1926, lxxviii, 136.
(4) Hewitt. Biochem. Journ., 1924, xviii, 161.
(121) Hewitt and Pryde. Biochem. Journ., 1920, xiv, 395.

(44) HILL, L. Lancet, 1928, ccxv (2), 802.
(2) HÖBER. Physichalische Chemie der Zelle und der Gewebe, Part II, 1924, 754.
(62) HÖBER. Pflüger's Arch., 1901, lxxxvi, 199.
(63) HÖBER. Ibid., 1903, xciv, 337.
(64) HÖBER. Biochem. Zeitschr., 1909, xx, 56.
(65) HÖBER. Pflüger's Arch., 1899, lxxiv, 246.
(114) HÖBER. Ibid., 1904, ci, 607.
(69) IRVING. Journ. Biol. Chem., 1926, lxviii, 513.
(33) ISHIKAWA. Jap. Journ. Med. Sci., 1928, iv, 205.
(182) JACKSON. Inanition and malnutrition. 1925, 322.
(102) JARISCH. Biochem. Zeitschr., 1922, cxxxiv, 163.
(120) KATSURA. Tohoku Journ. Exper. Med., 1926, vii, 382.
(67) KATZENELLENBOGEN. Pflüger's Arch., 1906, cxiv, 522.
(192) KELLY. Biochem. Journ., 1925, xix, 559.
(139) KING AND ARNOLD. Amer. Journ. Physiol., 1921, lix, 97.
(156) KOESSLER AND HANKE. Journ. Biol. Chem., 1924, lix, 889.
(148a) KOJIMA. (3 papers) Tohoku Journ. Exper. Med., 1929, xiii, 237.
(22) KOKAS AND GAL. Biochem. Zeitschr., 1928, ccv, 380.
(24) KÖRÖSY. Zeitschr. f. Physiol. Chem., 1913, lxxxvi, 356.
(37) KOTSCHNEFF. Pflüger's Arch., 1923, cci, 362.
(37a) KOTSCHNEFF ET AL. A series of papers Pflüger's Arch., 1926, ccxiv, 320.
(120a) KOTSCHNEFF. Pflüger's Arch., 1926, ccxiv, 343.
(34) KROGH. Anatomy and physiology of the capillaries, 1924, 247.
(128) LANDIS. Amer. Journ. Physiol., 1925, lxxv, 548.
(141) LANDIS. Ibid., 1928, lxxxiii, 528.
(95) LANGECKER. Arch. f. Exper. Path. u. Pharm., 1928, cxxxvi, 257.
(96) LASCH. Biochem. Zeitschr., 1926, clxix, 301.
(116) LASCH. Biochem. Zeitschr., 1926, clxix, 292.
(97) LASCH AND BRÜGEL. Arch. f. Exper. Path. u. Pharm., 1926, cxvi, 7.
(180) LAURENS. Physiol. Reviews, 1928, viii, 1.
(132) LEATHES AND RAPER. Monographs on Biochemistry. The fats. 1925.
(169) LEE, TEEL AND GAGNON. Proc. Soc. Exper. Biol. and Med., 1929, xxvii, 23.
(136) LOEVENHART. Amer. Journ. Physiol., 1902, vi, 331.
(13) LONDON. Abderhalden's Biologische Arbeitsmethoden iv, 1926, vi, 491, 544.
(14) LONDON. Zeitschr. f. Physiol. Chem., 1911, lxxiv, 322.
(35) LONDON. Pflüger's Arch., 1923, cci, 360.
(36) LONDON. Abderhalden's Biologische Arbeitsmethoden v, 1927, iv, 1921.
(110) LONDON AND DMITRIEW. Zeitschr. f. Physiol. Chem., 1910, lxv, 213.
(89) LONDON AND POLOWZOWA. Zeitschr. f. Physiol. Chem., 1908, lvi, 512.
(61) LOVATT-EVANS. Journ. Physiol., 1923, lviii, 22.
(42) LURJE. Pflüger's Arch., 1926, ccxii, 64.
(161) MCCALLUM AND MAGEE. Quart. Journ. Exper. Physiol., (in press).
(150) MCCARRISON. Studies in deficiency disease, 1921.
(100) MCCLENDON AND MEDES. Physical chemistry in biology and medicine, 1925, 202.
(45) MACLEOD, MAGEE AND REID. Unpublished results.

(171) MAGEE. Journ. Agric. Sci., 1924, xiv, 516.
(149) MAGEE, ANDERSON AND MCCALLUM. Lancet, 1929, 1, 5497.
(187) MAGEE, ANDERSON AND MCCALLUM. Quart. Journ. Exper. Physiol., 1928, xix, 171.
(194) MAGEE AND HARVEY. Biochem. Journ., 1926, xx, 885.
(54) MAGEE AND MACLEOD. Journ. Physiol., 1929, lxvii, xvii.
(55) MAGEE AND MACLEOD. Amer. Journ. Physiol., 1929, xc, 442.
(196) MAGEE AND REID. Journ. Physiol., 1927, lxiii, 97.
(40) MAGEE AND SOUTHGATE. Journ. Physiol., 1929, lxviii, 67.
(170) MAGNUS-LEVY. cit. LUSK, Science of nutrition, 1921, 381.
(109) MELLANBY, J. Journ. Physiol., 1927, lxiv, V.
(157) MAMMOSER AND BOYD. Proc. Soc. Exper. Biol. and Med., 1929, xxvi, 765.
(186) MENVILLE, BLACKBERG AND ANE. Proc. Soc. Exper. Biol. and Med., 1929, xxvi, 758.
(39) MIEKLE. Pflüger's Arch., 1928, ccxxi, 743.
(17) MOND. Ibid., 1924, ccvi, 172.
(111) MOND. Ibid., 1926, ccxv, 468.
(58) MOORE. Biochemistry, 1921, 127.
(183) MORGULIS. Fasting and undernutrition, 1923, 226.
(176) MOTTRAM, CRAMER AND DREW. Brit. Journ. Exper. Path., 1922, iii, 179.
(175) MURLIN. Amer. Journ. Obstet., 1917, lxxv, 943.
(164) MURRAY. Brit. Journ. Exper. Path., 1923, iv, 335.
(12) NAGANO. Pflüger's Arch., 1902, xc, 389.
(135) NEDSWEDSKI. Ibid., 1926, ccxiv, 337.
(15) NEVER. Ibid., 1928, ccxix, 554.
(197) ORR, J. B. Brit. Assoc. for Adv. Sci., Southampton, 1925, 204.
(165) ORR AND LEITCH. Iodine in nutrition. H.M.S.O., London, 1929, 51.
(179a) ORR, MAGEE AND HENDERSON. Journ. Physiol., 1924, lix, XXV.
(179b) ORR, MAGEE AND HENDERSON. Biochem. Journ., 1925, xix, 569.
(99) PETSCHACHER AND FELDER. Arch. f. Exper. Path. u. Pharm., 1928, cxxxiv, 212.
(20) PIERCE, OSGOOD AND POLANSKY. Proc. Soc. Exper. Biol. and Med., 1929, xxvi, 347.
(21) PIERCE, OSGOOD AND POLANSKY. Journ. Nutrition, 1929, i, 247.
(53) PURVES. Unpublished results.
(50) PUTNAM. Amer. Journ. Physiol., 1923, lxiii, 548.
(168) PUTNAM, TEEL AND BENEDICT. Amer. Journ. Physiol., 1928, lxxxiv, 157.
(79) RABINOVITCH. Amer. Journ. Physiol., 1927, lxxxii, 279.
(74) REID, E. W. Journ. Physiol., 1901, xxvi, 436.
(75) REID, E. W. Brit. Med. Journ., 1892, i, 1133.
(76) REID, E. W. Journ. Physiol., 1902, xxviii, 241.
(77) REID, E. W. Proc. Roy. Soc., 1899, lxv, 94.
(119) REID, E. W. Journ. Physiol., 1898, xxii, LVI.
(143) REID, E. W. Journ. Physiol., 1896, xx, 298.
(193) RICHARDS, GODDEN AND HUSBAND. Biochem. Journ., 1927, xxi, 971.
(146) ROGOFF AND STEWART. Amer. Journ. Physiol., 1926, lxxviii, 683.
(147) ROGOFF AND STEWART. Amer. Journ. Physiol., 1926, lxxix, 508.
(70) RÖHMANN AND NAGANO. Pflüger's Arch., 1903, xcv, 533.

(172) Root and Root. Arch. Int. Med., 1923, xxxii, 411.
(174) Rowe, Alcott and Mortimer. Amer. Journ. Physiol., 1925, lxxi, 667.
(184) Rowlands. Proc. Roy. Soc. Med., (Compar. Med. 41), 1927.
(185) Rowlands and Browning. Lancet, 1928, 1, 5448.
(173) Sandiford and Wheeler. Journ. Biol. Chem., 1924, lxii, 329.
(31) Sataké and Fujii. Tohoku Journ. Exper. Med., 1926, vii, 522.
(32) Sataké and Hirayama. Ibid., 1926, vii, 535.
(148) Sataké and Kojima. Amer. Journ. Physiol., 1929, xc, 505.
(154) Scott. Journ. Exper. Med., 1928, xlvii, 185.
(162) Sharpey-Schafer. The endocrine organs, Part 1, 1924, 20.
(48) Starling. Fluids of the body, 1909, 49.
(123) Stiven and Reid. Biochem. Journ., 1923, xvii, 556.
(189) Sun. Anat. Rec., 1927, xxxiv, 341.
(166) Taylor and Fine. Amer. Journ. Physiol., 1929, xc, 268.
(91) Török. Zentralb. f. Physiol., 1906, xx, 206.
(90) Traube. Pflüger's Arch., 1904, cv, 559.
(93) Traube. Biochem. Zeitschr., 1910, xxiv, 323.
(181) Underhill and Mendel. Amer. Journ. Physiol., 1927, lxxxiii, 589.
(3) Vérzar. Handbuch der normalen und pathologischen Physiologie, III and IV edited by Bethe, Embden, etc., 1929, p. 37 of reprint.
(101) Vérzar. Amer. Journ. Physiol., 1929, xc, 274.
(25) Vérzar and Kokas. Pflüger's Arch., 1927, ccxvii, 397.
(103) Vérzar and Kuthy. Biochem. Zeitschr., 1928, ccv, 369.
(104) Vérzar and Kuthy. Ibid., 1929, ccix, 265.
(105) Vérzar and Kuthy. Ibid., 1929, ccix, 281.
(158) Wagensteen. Arch. Surg., 1928, xvi, 606.
(68) Wallace and Cushny. Amer. Journ. Physiol., 1898, i, 411.
(11) Walsh and Ivy. Proc. Soc. Exper. Biol. and Med., 1928, xxv, 839.
(112) Weise. Arch. internat. de pharmacodyn., 1911, xxi, 77.
(10) White and Rabinovitch. Journ. Biol. Chem., 1927, lxxiv, 449.
(84) Wilson and Lewis. Journ. Biol. Chem., 1929, lxxxiv, 511.
(122) Winter and Smith. Journ. Physiol., 1922, lvii, 100.

HEMOGLOBIN, THE HEME PIGMENTS, AND CELLULAR RESPIRATION

M. L. ANSON AND A. E. MIRSKY

Laboratories of The Rockefeller Institute for Medical Research, Princeton, N. J., and the Hospital of the Rockefeller Institute for Medical Research, New York

Hemoglobin has long been studied as a carrier of oxygen in the blood of vertebrates. More recently (1885) a pigment related to hemoglobin was observed by MacMunn in the cells of a great variety of lower animals which have no hemoglobin either in their cells or body fluids. After long neglect, MacMunn's observations were recalled, and were confirmed and extended. In addition still another pigment was discovered which is related to hemoglobin and which in cells acts as a respiratory catalyst. A group of pigments called heme pigments and closely related to hemoglobin is accordingly now known. They exist in all aerobic cells where one of them, at least, plays an essential rôle in cellular oxidations. This review is concerned with the chemical properties and biological behavior of heme pigments. Hemoglobin will be included in the discussion because knowledge of the pigments is largely based on a wide study of hemoglobin and its derivatives. Hemoglobin itself is a compound of a colorless protein, globin, and an iron porphyrin pigment. In general the pigments formed by the combination of iron with the various porphyrins are called hemes.

The functions of the heme pigments have attracted investigators with very varied interests so that they have been studied from many different points of view. In organic chemistry their constitution has been of interest; in physiology their chemical properties were analyzed first in order to understand the respiratory functions of the blood, and more recently in connection with the problems of cellular respiration; in physics, at least in the case of one investigator, they have been studied because of their clearly defined spectroscopic properties; in pharmacology because of their reactions with drugs and poisons; and in natural history because of their wide distribution in nature.

These investigations of the heme substances may be considered to have been along four lines.

1. The investigations of the organic chemists (Hoppe-Seyler, Nencki, Piloty, Küster, Willstätter and Hans Fischer) on hemoglobin present a continuous development from the time of Hoppe-Seyler to the present. Although until recently the knowledge gained has been of relatively little physiological significance, the physiologist now has problems that will cause him to seek the aid of organic chemistry. How, for instance, do the heme-like pigments differ from the heme of hemoglobin, and how are the porphyrins of the hemes related to the porphyrins of chlorophyll? The similarity in constitution between heme and chlorophyll becomes more interesting to the physiologist now that he is aware that heme is a universal cellular constituent. Investigation of the porphyrins is at present very active, due mainly to Hans Fischer and his school. Only the bare outlines of these extensive and thorough investigations can be given in this review. Fischer has written several general accounts (1924, 1929a).

2. The physiologist has studied the intact heme molecule from the point of view of hemoglobin, for this was until recently the only heme substance of any known physiological significance. When the other heme pigments occurring in nature were discovered, the question of how globin modifies the properties of heme in hemoglobin was expanded into the general problem of how heme is influenced by combination with nitrogen compounds. Much of the information acquired in the study of hemoblogin (for instance the knowledge of the reactions between carbon monoxide and hemoglobin) was simply applied to other heme compounds.

3. Naturalists, or chromatologists, as they often called themselves (such men as Sorby, MacMunn and Lankester), interested in the colors of the whole animate creation, added the spectroscope to the equipment of the naturalist, and examined plant and animal pigments wherever they could find them. MacMunn discovered heme-like substances in the cells of animals from sponges to vertebrates. These investigators had, what is so often lacking in physiologists, an interest in all forms of life. On the other hand, they failed to carry out any effective experiments. Perhaps it was for these reasons that they failed to influence the physiological study of hemoglobin. Indeed, these researches of the chromatologists, which were confined to the nineteenth century, were not appreciated, and were nearly forgotten. Finally, however, it was the observations of MacMunn that led to the contemporary study of the cellular heme pigments.

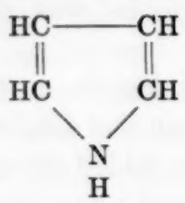
4. A fourth way of investigating heme pigments has recently been

developed by Warburg. The activity of these substances as respiratory catalysts is inhibited when they combine with carbon monoxide, but is restored when the carbon monoxide compound is dissociated by light. The behavior of heme catalysts can therefore be studied by measuring the oxygen uptake of systems exposed to known pressures of carbon monoxide and to light of definite wave lengths. By this method, heme pigments can be investigated in the living cell as well as in vitro.

Following the four points of view just outlined, this review will deal first with the chemistry of porphyrins, secondly with the chemistry of hemochromogen, thirdly with the distribution in nature of the heme pigments, and fourthly with the heme pigments as respiratory catalysts.

I. CHEMISTRY OF THE PORPHYRINS. The starting point for investigations of the porphyrins has usually been *hemin*, familiar as Teichmann's crystals. This is the hydrochloride of heme, the protein-free part of hemoglobin. Heme has a molecular weight of about 650 (Fischer and Hahn, 1913) which was measured directly by the effect of heme on the boiling point of pyridine. This estimate is the same as the minimum molecular weight calculated on the basis of the iron content of heme.

The problem of the constitution of heme was first studied by splitting it into its component parts and studying the parts. For this purpose heme has been broken down by oxidation, by reduction with acetic acid and hydrogen iodide, and by alkyl splitting. The products which were obtained are all derivatives of pyrrol

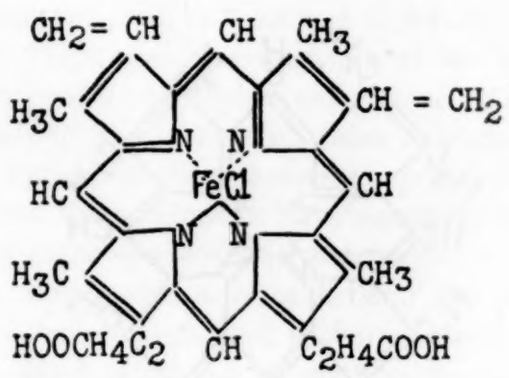


and have all been synthesized so that their structures are known. Some are pyrrol acids, others are pyrrol bases. A detailed study of these decomposition products showed that there are four pyrrol nuclei in heme, of which two are bases and two acids. Possible structures of heme were then proposed by Küster, Willstätter and Fischer. At the same time Fischer began his investigations on the synthesis of hemin.

1. *The Synthesis of Hemin.* Fischer's investigations were based on knowledge not only of the building blocks of heme, pyrrol derivatives obtained by a relatively vigorous decomposition, but also on an acquaintance with substances obtained from hemin by gentle treatment. The essential step in the preparation of these substances, the *porphyrins*,

is the splitting off of iron from the heme molecule. At the same time the side chains of the pyrrol nuclei are modified so that a number of different porphyrins can be prepared differing from each other in the constitution and arrangement of these side chains. Hematoporphyrin was the first porphyrin to be obtained in crystalline form (Nencki, 1900) and many have subsequently been crystallized, each having its characteristic crystal form, melting point and absorption spectrum. Some of the porphyrins obtained from heme occur in nature. The porphyrin whole structure is most closely related to that of heme is protoporphyrin with which iron can recombine to form a hemin indistinguishable from that prepared directly from hemoglobin. The porphyrins seem, indeed, able to combine with iron to form a series of hemes, which can be crystallized as hemins. The iron-porphyrin compound of aetioporphyrin (Willstätter, 1912), for instance, would be called aetioheme, its hydrochloride, aetiohemin. Aetioporphyrin is of special interest since it can be prepared from both hemoglobin and chlorophyll.

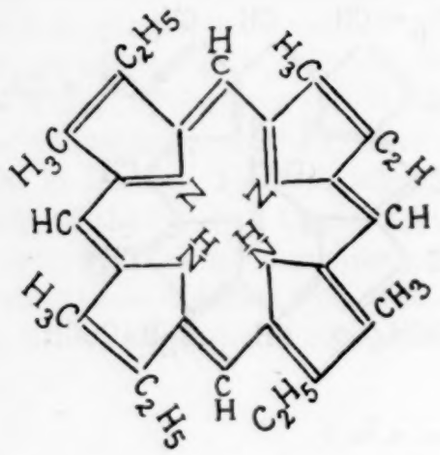
Because of the close relationship between the porphyrins and hemin, and because the pyrrol building blocks of hemin were already identified, Fischer's first step in the synthesis of hemin was naturally an attempt to join several pyrrol nuclei together to form a porphyrin. The first pyrrol compounds made did not have the properties of a porphyrin, even when they contained as many as four pyrrol nuclei; later, however, methods were discovered of combining four pyrrol nuclei in such a manner as to form a porphyrin (Fischer and Scheyer, 1924). Many porphyrins were synthesized, some of which had already been prepared from hemin but some of which were new. To make protoporphyrin it was necessary to introduce two unsaturated side chains. The addition of iron completed the synthesis (Fischer and Zeile, 1929). Fischer's formula for hemin is:



There are several points of biological interest to be mentioned in connection with the synthesis of hemin: The difficulty in introducing the unsaturated side chains was that the porphyrin was relatively inert chemically. To render it more active, the porphyrin was converted by adding iron into the corresponding heme to which side chains could then be joined. Iron was then withdrawn, alterations were made in the side chains to make them correspond to those of protoporphyrin, and finally iron was restored. Apparently an iron porphyrin is more active than a simple porphyrin. The free porphyrins which are found in nature are perhaps mainly excretory products.

In the synthesis of hemin, isomerism also presents a matter of biological interest. The hemin of hemoglobin is one of a number of isomers. Of the porphyrins now known to occur in nature some (viz. oöporphyrin, the porphyrin of egg shells) have the same configuration as the porphyrin of hemoglobin (Fischer and Kögl, 1923) and others (viz., turacin, a copper porphyrin present in the feathers of certain birds) a different configuration (Fischer and Hilger, 1923). The natural porphyrins now known are all constructed after the manner of two of the four possible arrangements. How the porphyrins of the cellular heme pigments are to be classified in this respect is not yet known.

2. *Heme Pigments and Chlorophyll.* In the study of the porphyrins the goal of the organic chemist has been not only the synthesis of hemin but also an understanding of the relationship between hemoglobin and chlorophyll. Before anything was known about the structure of either hemin or chlorophyll, suggestions were made that they had much in common. The first experimental evidence for the supposition was the preparation of a porphyrin from chlorophyll (Hoppe-Seyler, 1879, 1880, 1881). The nature of the relationship became clearer when Willstätter and Stoll (1913) prepared aetioporphyrin from both chlorophyll and hemoglobin.



Though this porphyrin is closely related to hemin, lacking only two unsaturated side chains and an iron atom, its relation to chlorophyll is obscure. Willstätter and Stoll considered it unlikely that chlorophyll itself contains the porphyrin nucleus, but rather that porphyrin is formed during decomposition. They were able to separate the decomposition of chlorophyll into a number of definite stages, each of which was characterised by the development of a crystalline substance. Of these the *chlorines* form an important group, marking a relatively early stage in the breakdown of chlorophyll. When the chlorines themselves break down, porphyrins are formed. The recent studies of Hans Fischer and his pupils throw a new light on the relationship between these two groups of substances, for they have been able to prepare chlorines also from porphyrins (Fischer, 1929b). Reducing a porphyrin with an alcoholate converts it into a chlorine, though by this simple procedure only a small yield is obtained. Since combination with iron renders a porphyrin much more active, the reaction was tried on various hemes (i.e., iron porphyrins), iron being subsequently removed. Chlorines were in this way more easily prepared. Because of the near relationship between chlorines and porphyrins, Fischer thinks there is no longer any doubt that chlorophyll contains the porphyrin nucleus itself or a system closely related to it. It would be a remarkable fact if two substances of such importance in cellular metabolism both contained the porphyrin nucleus, the one, chlorophyll, associated with the formation of sugar, the other, heme, associated with the combustion of sugar.

II. Chemistry of hemochromogens. The porphyrin substances active in cellular respiration are not simply porphyrins and probably not merely hemes (i.e. iron-porphyrins); the respiratory catalysts are probably hemochromogens, and the cellular heme pigments actually visible are certainly hemochromogens. Since hemochromogens seem to play an important part in cellular metabolism their chemistry is of considerable biological interest and will be dealt with in detail.

1. *Spectroscopic Properties.* Investigation of the hemochromogens has been largely dependent on their spectroscopic properties. In fact much of the knowledge available of all those substances mentioned in this review—porphyrins, chlorines, hemes, etc.—was obtained with the spectroscope because these pigments have unusually sharp absorption bands and because exact spectroscopic measurements are easily made. It is not only the exact position of each band but also the arrangement of the bands, their pattern, that is characteristic of each group of these substances. Each group can be identified by its own absorption

pattern. It was for this reason that Hans Fischer supposed he had prepared chlorines from porphyrins, the absorption bands of the new substances being arranged in the same pattern as those of the "natural" chlorines prepared from chlorophyll by Willstätter. Nearly every step in the synthesis of porphyrins was guided by the spectroscope, though in every case information of other kinds was subsequently gathered. That spectroscopic knowledge should be reliable and its significance profound is amply explained by modern physics. It is naturally desirable to make spectroscopic measurements as precise as possible in order to distinguish between closely related compounds. There are, for instance, isomeric porphyrins whose bands lie approximately in the same position, indeed to within 10Å[1], so near together that it seemed necessary to resort to melting point determinations to distinguish them (Fischer and Schormüller, 1929). Though 10Å is the limit of accuracy attainable for these bands with an ordinary spectroscope, they could perhaps be measured with an error not greater than 1 or 2 Å with the reversion spectroscope (Hartridge, 1912). The absolute amount of light absorbed at any particular wave-length should, moreover, also be measured, for only by doing so can a substance be completely characterized spectroscopically.

The importance of spectroscopic investigation of the heme pigments can only be fully perceived when one attempts to study these substances within the living cell. In chemical physiology it is frequently a most perplexing problem to find a way of observing and measuring the chemical changes which occur in living cells without changing the cell at the same time. The situation closely resembles that in astronomy where the chemistry of a body can be studied only from a distance. Chemistry of the stars has been made possible by the use of the spectroscope; in the same way, the chemistry of cells, in so far as it is concerned with substances having clearly defined spectroscopic properties, can be investigated. In the spectroscopic study of cells, as of stars, interpretation of observations depends upon an acquaintance with the spectroscopic properties of the substances involved. Consequently in discussing the chemistry of hemochromogen, emphasis must be laid on spectroscopic properties.

Kirchoff's discovery in 1859–60 of the explanation of Frauenhofer's lines in the solar spectrum, pointed the way to a knowledge of the chemical composition of the sun and stars; in the same year Bunsen applied

[1] Angstrom units.

the principles of spectrum analysis to the examination of terrestrial matter. Two years later Hoppe-Seyler observed the absorption spectrum of hemoglobin (Hoppe-Seyler, 1862). Hoppe-Seyler's paper was called to the attention of Stokes, the eminent physicist, who later wrote, "I had no sooner looked at the spectrum, than the extreme sharpness and beauty of the absorption bands of blood excited a lively interest in my mind, and I proceeded to try the effect of various reagents." The result was a study that has been the basis for all subsequent spectroscopic investigation of the heme pigments (Stokes, 1863–64). The experiments on hemoglobin, for instance, demonstrated that the difference in color between arterial and venous blood is essentially due to the difference in color between oxygenated and reduced hemoglobin.

When Stokes reduced[2] an alkaline solution of what we now call heme, he discovered a substance having two intense, sharp, absorption bands, and when he reduced a solution of hemoglobin previously warmed in alkali, the same spectrum was obtained. The substance with this absorption spectrum was called hemochromogen by Hoppe-Seyler[3] (1870).

2. *Chemical Nature of Hemochromogens* (Anson and Mirsky, 1925a, 1928). The hemochromogens are a series of compounds with the same absorption spectrum pattern but with absorption bands in different positions. A hemochromogen is formed when reduced heme combines with one of a number of nitrogen substances. Reduced heme, prepared in the absence of a nitrogen compound, is unmistakably different from a hemochromogen: in the region in which hemochromogen has intense, sharp absorption bands, reduced heme shows weak, diffuse absorption; under conditions under which hemochromogen is soluble, reduced heme is insoluble; and, of perhaps greatest interest biologically, combination with a nitrogen substance makes heme a much more effective oxidative catalyst. The differences between hemochromogens formed from the same reduced heme are due to the different nitrogen compounds with which the reduced heme is combined. Many, though by no means all, nitrogen compounds can combine with reduced heme to form hemo-

[2] Stokes used an ammoniacal reducing agent.

[3] Hoppe-Seyler substituted this name for that used by Stokes because he believed that as the person who had first isolated the substance in crystalline form he had the right to name it, though later he admitted having been mistaken in claiming to have crystallized hemochromogen. (Hoppe-Seyler and Thierfelder, 1893.)

chromogens. Cyanide, ammonia, amines, proteins, pyridine, nicotine, etc., have this property. The compounds appear to have only nitrogen in common. A hemochromogen is named by using the name of its nitrogen substance as a prefix, as for example, pyridine-hemochromogen, ammonia-hemochromogen. The hemochromogen most frequently observed is that prepared directly from a hemoglobin solution by adding alkali and a reducing agent (sodium hydrosulfite, for instance). The nitrogen substance which this hemochromogen contains is denatured globin, and it is therefore called globin-hemochromogen. If globin is separated from heme before adding the reducing agent and heme then reduced with sodium hydrosulfite, reduced heme, not hemochromogen is obtained. If globin is now added it combines with reduced heme so that the absorption spectrum of heme disappears and that of globin-hemo-chromogen takes its place.

The ability to form hemochromogen seems to be a general property of hemes (iron-porphyrins). Mesoheme and aetioheme, for instance (formed by the union of iron with mesoporphyrin and aetioporphyrin respectively) combine with nitrogen substances to form mesohemochromogens and aetiohemochromogens (Willstätter, 1912).

3. *History of Hemochromogen.* The account of hemochromogen just given differs so much from the views of the chemistry of hemochromogen until recently generally accepted, that some historical statement is needed. Since the discovery of hemochromogen by Stokes in 1864 the substance has always been identified by its absorption spectrum. That hemochromogen was simply reduced heme was accepted as a matter of fact by Stokes and subsequent investigators, though we now see that it was a theory based on the fact that when Stokes reduced heme he obtained hemochromogen. Stokes was unaware of the fact that when heme was reduced it also combined with ammonia in the reducing system used (for Stokes used ammoniacal reducing agents), to form a hemochromogen. Indeed it was soon found by Hoppe-Seyler that some reducing agents did not form hemochromogen from heme unless ammonia, albumin, or some other "organic" substance was added, which substance, it was thought, enhanced the reducing action of the system (Hoppe-Seyler, 1878). Consequently some reducing agents, particularly hydrazine hydrate (a nitrogenous one, we may note) were held with favor as being the proper reducers for heme. When hemochromogen was not formed it was thought that no reduction had occurred; reduced heme had not yet been discovered. In 1893 Bertin-Sans and Moitessier discovered reduced heme and realized that this turns into one or other of the hemochromogens "by the action" of ammonia, amines, and proteins (Bertin-Sans and Moitessier 1893a, 1893b). Whether "by the action" was meant an effect on the reducing agent or on the reduced heme was not stated. Subsequent investigators (Laidlaw, 1904) thought of the experiments of Bertin-Sans and Moitessier as having confirmed Hoppe-Seyler's view that a weak reducing agent is rendered effective by the pres-

ence of protein. As for the existence of reduced heme as distinct from hemochromogen, this was confirmed by some investigators (Menzies, 1894) and not by others (Laidlaw, 1904, Hasselbalch, 1909). (Dilling appears to have agreed with Hasselbalch.) Dilling (1910) went so far as to show that the hemochromogens crystallized from pyridine, piperidine and a number of similar bases differ from each other. His interpretation of the differences observed was that hemochromogen is an acid that can combine with various bases to form a series of esters.[4] Dilling thought that only secondary differences between the hemochromogens, such as the small differences in the positions of absorption bands, are due to combination of these bases "with the *pre-existing* hemochromogen radicle."[5] This view that hemochromogen is essentially reduced heme continued to be universally held as is illustrated by the fact that in 1923 Hans Fischer and Schneller (1923a), being able to add iron to porphyrins, tried to make hemochromogen simply by adding iron in the absence of oxygen. Finally it was definitely shown by Anson and Mirsky (1925a, 1928) that hemochromogen is not simply free reduced heme but is a compound of heme and a nitrogen group and that the particular hemochromogen prepared from hemoglobin is a compound of globin and heme. These conclusions are now generally accepted. (Hill and Holden, 1926; Roche, 1929.)

4. *Hemochromogens as Definite Compounds.* The clearly defined spectroscopic properties of the hemochromogens indicate that these substances are not vague complexes. Additional evidence that they are definite compounds is the fact that from solutions of pyridine-hemochromogen and some similar hemochromogens, crystals can be prepared (Donogany, 1893; Dilling, 1910) which by examination with a microspectroscope are proven to be hemochromogen crystals (Anson and Mirsky, 1928). Not all hemochromogens, however, have been crystallized.

5. *Shifts of Absorption Bands.* Under given conditions, the positions of the absorption bands of a hemochromogen prepared from a definite nitrogen compound are constant and exactly reproducible. In some cases, however, large shifts in position can be produced which are associated with precipitation of the hemochromogen (Anson and Mirsky, 1925a). These changes in position are probably caused by aggregation of the hemochromogen molecules (Keilin, 1926). In general, aggregation of a pigment causes its absorption bands to shift towards the red end of the spectrum (Stenger, 1888, Svedberg, 1909). The correlation between dispersion and spectrum is easily observed with globin-hemo-

[4] Dilling's theory of ester formation is disproved by the facts that ester formation is impossible in the strongly alkaline solutions in which hemochromogens exist, that cyanide, which forms a typical hemochromogen is not a base and that hemes not possessing free carboxyl groups can form hemochromogens.

[5] The italics are ours.

chromogen (Mirsky and Anson, 1929a). If this hemochromogen is precipitated by salting out in alkaline solution or by neutralization, the band shifts some twenty-five Å toward the red end of the spectrum. The shift is always in this direction and may in some hemochromogens be as much as 170 Å. (Keilin, 1926.)

6. *Hemochromogen Equilibria* (Anson and Mirsky, 1925a, 1928). In a hemochromogen the nitrogen compound is loosely combined with reduced heme so that some uncombined nitrogen compound is always present, there being an equilibrium between reduced heme, free nitrogen compound and hemochromogen.

$$\text{Reduced heme} + \text{Nitrogen compound} \rightleftharpoons \text{Hemochromogen}$$

This equilibrium is closely analogous to the equilibrium formed when hemoglobin combines with oxygen for there, too, some free oxygen is always present.

$$\text{Hemoglobin} + \text{Oxygen} \rightleftharpoons \text{Oxyhemoglobin}$$

In the hemoglobin system the existence of an equilibrium is demonstrated by the fact that although only one molecule of oxygen is combined with one atom of iron in oxyhemoglobin, to convert all of the reduced hemoglobin into oxyhemoglobin, more than the equivalent amount of oxygen must be added. An excess of oxygen must be present to drive the above equilibrium to the right. Similarly, an excess of nitrogen compound must be added to reduced heme to convert all of it into hemochromogen, to drive the hemochromogen equilibrium to the right.

The analogy may be followed even further. Hemoglobin combines with carbon monoxide as it does with oxygen, a difference being that much less free carbon monoxide than oxygen is needed to obtain combined hemoglobin. The affinity of hemoglobin for carbon monoxide is, as is well known, greater than for oxygen. Similarly, the affinity of reduced heme for cyanide, for instance, is greater than for pyridine or ammonia. When hemoglobin is exposed to oxygen and carbon monoxide at the same time, the two gases compete for the hemoglobin.

$$HbO_2 + CO \rightleftharpoons HbCO + O_2$$

In the same way, different nitrogen compounds compete for reduced heme.

$$N_1 \text{ hemochromogen} + N_2 \rightleftharpoons N_2 \text{ hemochromogen} + N_1$$

Hemoglobin and hemochromogen equilibria are both affected by temperature and acidity. Hemochromogen when heated is dissociated into reduced heme and nitrogen substance. The affinity of reduced heme for a nitrogen substance increases as the solution becomes more alkaline. The precise effect of hydrogen ion concentration depends, to some extent, on the nature of the nitrogen substance, some requiring a definitely alkaline solution to combine with reduced heme; others being able to combine in approximately neutral solutions.

In studying hemoglobin and hemochromogen equilibria, the same methods can sometimes be used. Thus, ammonia-hemochromogen dissociates in a vacuum into reduced heme and free ammonia as does oxyhemoglobin into hemoglobin and oxygen. Although the methods originally used for the study of hemoglobin equilibria were gasometric and colorimetric, more recently spectroscopic methods have been used too. The equilibrium in which oxygen and carbon monoxide compete for hemoglobin can be studied quantitatively with a spectroscope simply by measuring the changing positions of an absorption band (Hartridge, 1912). The analogous hemochromogen equilibrium in which two nitrogen substances compete for reduced heme can be observed in precisely the same manner.

7. *Stoicheiometry of Hemochromogen.* The equilibria just described complicate the problem of finding the number of molecules of a nitrogen compound combining with one molecule of reduced heme to form a hemochromogen molecule. Analyses of "isolated" hemochromogens were made before hemochromogen equilibria were discovered, but we have no assurance that the substance analyzed was pure hemochromogen (von Zeynek, 1898, 1910; Pregl, 1905). Since a hemochromogen exists only in the presence of free nitrogen substance, it is hardly possible to isolate the hemochromogen uncontaminated by free nitrogen compound or by free reduced heme. It is also possible that a nitrogenous base in addition to acting as a nitrogen compound to form a hemochromogen, might as a base combine with the acid groups of heme. Analysis of "isolated" hemochromogen would include both forms of nitrogenous base.

If in the equilibrium

$$\text{Reduced heme} + \text{Nitrogen compound} \rightleftharpoons \text{Hemochromogen}$$

the affinity for nitrogen compound is so great that the amount of *free* nitrogen compound is negligible compared with the amount of *combined* nitrogen compound, then the *total* nitrogen compound (which can be

known) will be practically the same as that combined. Hill (1926, 1929) found that the addition of two molecules of pyridine is required to convert one molecule of reduced heme into hemochromogen. He believes that in his experiments the free pyridine was negligible, that all the pyridine added was combined with heme, and that therefore hemochromogen contains two nitrogen groups per heme. It is our opinion (Mirsky and Anson, 1929a; Anson and Mirsky, 1930b) that it was not proven that in Hill's experiments the free pyridine was negligible, for the aggregation of both reduced heme and pyridine hemochromogen complicates to an unknown extent the study of the pyridine hemochromogen equilibrium. If all the pyridine added was not combined with heme, then pyridine hemochromogen contains less than two pyridine groups per heme. From the study of the reactions of heme with cyanide the simplest conclusion is that hemochromogen contains one nitrogen group per heme.

8. *Reactions of Heme with Cyanide* (Anson and Mirsky, 1928, 1930b). It has long been known that cyanide reacts with reduced heme to form a pigment with a two-banded spectrum similar to, but definitely different from that of ordinary hemochromogen (Oppenheimer's Handbuch, 1909). A closer study of this reaction has shown that just one molecule of cyanide reacts with one molecule of reduced heme to form a compound which has a typical hemochromogen spectrum, and then still more cyanide reacts with this hitherto unobserved first cyanide compound of reduced heme to form the familiar second cyanide compound. Hill (1929) who has confirmed these results, has also shown that the second cyanide compound contains two cyanide groups per heme. The experiments with cyanide are simpler than those with pyridine because cyanide has a much greater affinity for reduced heme than pyridine so that it is relatively easy to make the free cyanide negligible.

Experiments on the reactions of cyanide with globin hemochromogen show that cyanide reacts with globin hemochromogen to form a compound of globin hemochromogen and cyanide. In this reaction cyanide does not compete with or displace globin and so is not reacting like a typical nitrogenous substance. Cyanide can then further react with cyanide-globin hemochromogen to form the same second cyanide compound of reduced heme which is formed in the absence of globin.

We have, then, these facts, that the first cyanide compound of reduced heme contains one cyanide group per heme and has a typical hemochromogen spectrum, that the second cyanide compound does not have a typical hemochromogen spectrum, and that cyanide can combine

with hemochromogen without reacting as a typical nitrogenous substance. So it seems simplest, for the present, at least, to conclude that in the absence of other nitrogenous substances, cyanide first combines with heme to form a typical hemochromogen containing one nitrogen group per heme and then combines with this cyanide hemochromogen just as it does with globin hemochromogen, this second reaction having nothing to do with hemochromogen formation.

9. *Combination of Heme with Proteins.* Proteins, as nitrogenous substances, combine with reduced heme to form hemochromogens. Denatured globin combines with reduced heme to form globin-hemochromogen, native globin combines with heme to form hemoglobin.

a. *Proteins as complicated nitrogen substances containing a large number of free nitrogen groups.* One cannot know in advance which and how many of these groups combine with reduced heme. A single molecule of denatured globin (hypothetical molecular weight 16,700) can convert at least 10 molecules of heme into hemochromogen, an estimate made by adding more and more reduced heme to denatured globin until there appears in addition to the hemochromogen bands, the characteristic absorption bands of reduced heme (Anson, 1926; Mirsky, 1926; Anson and Mirsky, 1928; Holden and Freeman, 1929). It takes much more edestin than globin to convert a given amount of heme into hemochromogen and more zein than edestin. The low hemochromogen-forming capacity of zein may be connected with its low diamino acid content. (Anson and Mirsky, 1928.) Similar differences between other proteins have been observed (Holden and Freeman, 1929).

b. *The difference between globin-hemochromogen and hemoglobin.*[6] One difference between these two compounds of globin and heme is that hemochromogen is a compound of heme and denatured globin, while hemoglobin is a compound of native globin (Anson and Mirsky, 1925d; confirmed by Hill and Holden, 1926; Haurowitz, 1929; Roche, 1929, and others). There may be still other differences between hemoglobin and globin-hemochromogen. It is not yet known whether these two substances have the same molecular weight or in precisely what way heme and globin are combined in either hemochromogen or hemoglobin. It is known, however, that in neither compound is the linkage through the unsaturated side chains of heme (Hill and Holden, 1926).

[6] Based on the old theory that hemochromogen and reduced heme are identical was the further theory which, so far as we know, had never been questioned until 1925, that when hemoglobin is converted into hemochromogen, globin is split off from the iron pyrrol complex, heme.

c. *The significance for protein chemistry of combination with heme.* Since hemoglobin is a compound of heme with native globin, and hemochromogen one with denatured globin, we have here a specific chemical criterion of whether globin combined with heme is native or denatured, an indicator as it were, of the state of the globin. The globin prepared by the classical method of Schulz (1898) is denatured globin not, as was originally supposed, the native globin of hemoglobin (Anson and Mirsky, 1925d, confirmed by Hill and Holden, 1926). After this was made clear, mehods were devised for preparing native globin (Hill and Holden, 1926; Anson and Mirsky, 1930a). Though the remarkable physico-chemical differences between native and coagulated (or denatured) proteins are at once obvious, little is known of the underlying structural differences. Recent experiments indicate that the coagulation of hemoglobin, whether by heat, acid, or surface action, is reversible, for from completely coagulated hemoglobin a crystalline protein can be prepared having the same physico-chemical properties (solubility, coagulability, etc.) as the original native hemoglobin. There must also be "reversal" of some specific chemical groups, for the "reversed" protein combines with heme to form a hemoglobin whereas the coagulated protein combines with heme to form a hemochromogen. These recent investigations (Anson and Mirsky, 1925d, 1929b, 1930a; Mirsky and Anson, 1929b, 1930), on the reversal of protein coagulation can only be referred to here, but they suggest ways in which the types of linkage between heme and globin can be used in studying some important protein reactions. Holden and Freeman (1929) claim that coagulable proteins can form hemochromogens only when they are denatured.

d. *The "synthesis" of hemoglobin.* Hemoglobin has been formed from heme and globin. Bertin-Sans and Moitessier (1892, 1893b) who first did this were careful to ascertain by sensitive spectroscopic tests that the globin they used was free of heme and the heme free of globin. From such globin and heme, methemoglobin, reduced hemoglobin and oxyhemoglobin were prepared. Bertin-Sans and Moitessier tried to prepare hemoglobin crystals "which would give a definite sanction to their point of view," that they had in fact prepared hemoglobin, but in this respect they were unsuccessful. The synthesis has been repeated several times (Menzies, 1915; Anson and Mirsky, 1925a; Hill and Holden, 1926) and since then synthetic hemoglobin and methemoglobin have been crystallized (Anson and Mirsky, unpublished experiments).

10. *Hemochromogen Reactions with Carbon Monoxide and Oxygen.* a.

Carbon monoxide. Hemochromogens form loose combinations with carbon monoxide in much the same way that hemoglobin does.

$$\text{Hemochromogen} + \text{CO} \rightleftharpoons \text{CO Hemochromogen}$$

In the case of globin-hemochromogen this equilibrium (in the dark) is exactly described by the simple mass law equation. (Anson and Mirsky, 1925a.)

$$\frac{[\text{Hemochromogen}]\,[\text{CO}]}{[\text{CO Hemochromogen}]} = \text{K}$$

b. *Effect of light on carbon monoxide-hemochromogen.* The remarkable discovery of Haldane and Smith (1896) that light tends to dissociate carbon monoxide-hemoglobin has been found applicable also in the case of carbon monoxide-hemochromogen (Anson and Mirsky, 1925a Krebs, 1928). The combination of reduced heme itself with carbon monoxide is unstable in the light, but the instability is greatly increased when a nitrogen substance is joined to reduced heme, that is to say, carbon monoxide hemochromogen is more sensitive to light than is reduced carbon monoxide heme (Krebs, 1928).

The reactions with carbon monoxide are of fundamental importance for investigating the rôle of heme as a respiratory catalyst in the cell, for the CO compounds of heme are in general catalytically inactive and the CO compounds of the cellular heme pigments are extraordinarily sensitive to light. This sensitivity is closely approached by the substance formed when methyl carbylamine[7] combines with carbon monoxide-hemoglobin (Warburg, Negelein and Christian, 1929). Combining with this simple cyanide derivative renders carbon monoxide-hemoglobin several thousand times more sensitive to light. It is not known in this case whether carbylamine acts as a nitrogen substance, in the same manner as when combining with reduced heme to form a hemochromogen.

c. *Oxidation of hemochromogen.* Though hemochromogen, like hemoglobin, combines loosely with carbon monoxide, unlike hemoglobin, it is not known to combine loosely with oxygen. It reacts with oxygen and other oxidizing agents to form a substance analogous to methemoglobin, the oxide of hemoglobin. Hemoglobin and methemoglobin form a reversible oxidation-reduction system (Conant, 1923). When re-

[7] Methyl carbylamine ($CH_3N = C$) was used instead of cyanide ($HN = C$) because the latter is an acid.

duced hemoglobin, hemochromogen and reduced heme are formed from their oxides, one equivalent of hydrogen per atom of iron is required (Hill and Holden, 1927). The influence of its nitrogen substance on the tendency of a hemochromogen to oxidize is not known.[8] In general the conditions affecting the autoxidation and oxidation of the heme compounds await investigation. In the past observations on the so-called autoxidation of hemochromogens have been made only in strongly alkaline solutions containing oxidized products of reducing agents.

10. *Parahematin and hematin.* (Keilin, 1926.) The oxide of hemochromogen, the compound formed when a nitrogen compound combines with oxidized heme, is known as parahematin. It has a characteristic absorption spectrum of two rather diffuse bands. Perhaps the difficulty in recognizing the less sharply defined spectrum of parahematin accounts for the fact that knowledge of hemochromogen came earlier than that of parahematin. The concept of loose combination of reduced heme with nitrogen compounds to form hemochromogen can be extended with little change to oxidized heme with which the same nitrogen compounds form a series of parahematins. Changes in acidity may affect differently the combinations of reduced and oxidized hemes with nitrogen compounds. Globin-parahematin dissociates completely when a faintly alkaline solution is made tenth normal, in which solution globin-hemochromogen would scarcely dissociate at all (Keilin, 1926). On the other hand, according to Holden and Freeman (1929) denatured egg albumin forms a parahematin (as well as a hemochromogen) in normal sodium hydroxide, a parahematin which dissociates as the hydroxyl ion concentration is decreased.

The globin compound of oxidized heme has been known for some time (Arnold, 1900; van Klaveren, 1901) as cathemoglobin:[9] globin-para-

[8] Recently Conant and Tongberg (1930) have studied the oxidation-reduction potential of the reduced heme-oxidized heme system and have discovered that combination with nitrogen groups influences the potential. The calculations of oxidation-reduction potentials from the electromotive force measurements is made uncertain by the fact that the oxidation-reduction equilibrium is influenced to an unknown extent by the aggregation of the pigments (as Conant and Tongberg realized) and also by the fact that the relative extents to which pyridine combines with reduced and oxidized heme under the conditions of the experiments are not known. The complication of the pyridine-hemochromogen equilibrium by the aggregation of the pigments has already been considered in connection with spectroscopic experiments (Mirsky and Anson, 1929a; Anson and Mirsky, 1930b).

[9] It is curious to note that Dilling thought that when cathemoglobin is reduced to hemochromogen, globin is separated from reduced heme—so firmly established was the idea that hemochromogen is reduced heme.

hematin would now be a better name, the name indicating that it is one member of a series of compounds. The name hematin has been used for about a century(Berzelius, 1840) often in a vague way, for a number of hemoglobin derivatives in which globin was supposed to be separated from heme, and the same name was used later for a solution of hemin. The substances obtained when an excess of acid or alkali is added to hemoglobin are known as "acid hematin" and "alkaline hematin" respectively and a neutralized form as "neutral hematin." We have seen that "neutral hematin" is not free hematin and is now known as parahematin. There has never been any proof that the heme in acid hematin and alkaline hematin prepared by adding acid and alkali to hemoglobin is in the same state as in a solution of pure crystalline hemin, and it is to avoid the confusion of calling different things by the same name that the pure iron-porphyrin is designated as heme. There can be little doubt, moreover, that the heme in solutions of acid or alkaline hematin is not free, for free heme is insoluble in acid and the spectrum of alkaline hematin differs from that of free heme in alkali. The heme of acid and alkaline hematin is neither free nor in clearly defined compounds such as hemochromogen and parahematin but probably occurs in the form of colloidal complexes with globin (for alkaline hematin, see Wu, 1926; for acid hematin see Keilin, 1926).

III. The Heme Pigments in Nature. 1. *The Relations Between the Heme Pigments.* The study of the spectra and other properties of known heme compounds *in vitro* with which this review has so far been concerned provides the essential basis for the knowledge that heme compounds exist universally in nature, in all aerobic cells, and that one of them at least plays an essential rôle in the catalysis of biological oxidations. Hemoglobin itself is not a catalyst of oxidations but a passive carrier of oxygen and it is not universally distributed. It is present in all vertebrates. Outside of the vertebrates, however, hemoglobin is found only here and there, sometimes in the blood and sometimes in the tissues, of totally unrelated groups of animals. For instance, hemoglobin exists in the larvae of a few insects but in no other insect larvae and in no adult insect and it is found in a star fish but in no other similar animals. Apparently a very peculiar iron compound has arisen independently in nature many times.

In addition to hemoglobin there occur occasionally in nature a few other pigments which are related to hemoglobin in that they either are hemochromogens or can easily be converted into hemochromogens. Helicorubin is found in snails (Dhéré and Vegezzi, 1917a, 1917b, 1919;

Dhéré and Baumeler, 1926), actineohematin in the actiniae or sea anemones (MacMunn, 1885b) and chlorocruorin in certain worms (Milne Edwards, 1838; Lankester, 1867, 1870; Krukenberg, 1880 and 1882; MacMunn, 1885a, 1889a; Griffiths, 1892a, 1892b). The hemochromogens obtained from these pigments are not identical spectroscopically with the hemochromogen prepared from hemoglobin. *A priori* such differences may be due either to differences in the iron-porphyrin nuclei or to differences in the nitrogenous substances to which the iron-porphyrin nuclei are attached. It is possible to decide between these two possibilities by displacing the unknown nitrogenous substances with a known nitrogenous substance such as pyridine and comparing the spectrum of the resulting hemochromogen with that of pyridine hemochromogen made from the heme of hemoglobin. When this is done actineohematin and helicorubin yield the same pyridine hemochromogen as does the heme of hemoglobin (Anson and Mirsky, 1925b), while chlorocruorin yields a pyridine hemochromogen with bands distinctly further to the red (Fox, 1926a, 1926b). The heme of chlorocruorin can be converted not only into a hemochromogen but also into the other typical heme derivatives. There are then to be found in nature a number of true heme pigments, like hemoglobin of limited and haphazard distribution. Some of them, like actineohematin and helicorubin, yield the same heme as hemoglobin. Others, like chlorocruorin, yield a different heme.

2. *The Universality of Heme.* Of much wider distribution than any of the pigments so far mentioned is the histohematin of MacMunn (1886, 1887), which is related to and convertible into hemochromogen. MacMunn observed the sharp four-banded spectrum of histohematin in a great variety of animal tissues. For instance, in many echinoderms, molluscs, insects and worms as well as in the higher animals. Most of the animals in which histohematin was shown to exist do not contain hemoglobin. The important and easily verifiable observations of MacMunn were neglected for a generation because of the opposition of a great authority, Hoppe-Seyler (Levy, 1889; Hoppe-Seyler, 1890a, 1890b; MacMunn, 1889b, 1889c, 1890). Recently the study of histohematin has been revived by Keilin (1925, 1927) who has shown that the distribution of the pigment is even wider than MacMunn supposed (see also Bierich, Rosenbohm and Kalle, 1927; Yaoi and Tamiya, 1928; Yaoi, Tamiya and Nakahara, 1928). It is present in plants and in unicellular organisms such as yeasts or bacteria as well as in animal tissues. Keilin accordingly substituted for the name histohematin the more general name cytochrome.

The question naturally arose whether the more widely distributed heme-like pigments have the same iron-porphyrin nucleus as the occasional hemoglobin or the occasional chlorocruorin. Baker's yeast and the wing muscles of bees (which do not contain hemoglobin) were accordingly extracted with pyridine, the purpose being as with helicorubin and chlorocruorin to displace the unknown nitrogenous substances with pyridine. From both yeast and muscle two different pyridine hemochromogens were obtained, one indistinguishable from the pyridine hemochromogen derived from hemoglobin, the other similar to the hemochromogen derived from chlorocruorin (Anson and Mirsky, 1925b). It would be desirable to repeat these experiments with the new method now available (Anson and Mirsky 1930a) and first to separate beyond doubt the hemes from the unknown nitrogen compounds. More recently crystalline hemin identical or at least isomeric with the hemin from hemoglobin has actually been obtained from yeast (Fisher and Schwertel, 1928).

Hemoglobin, it now appears, may no longer be regarded as the preeminent heme pigment, nor is its haphazard distribution now so mysterious. Hemoglobin is merely an occasional specialized derivative of an iron substance, heme, which is much more widely distributed. Similarly the nucleus of the rare chlorocruorin, or a heme very like it, is present in aerobic cells generally. It would be interesting to know whether hemocyanin, the copper pigment which carries oxygen in the bloods of some crustacea, is also an occasional derivative of a universal copper compound. There is evidence (Phillipi, 1919) which still requires confirmation, that the pigmented part of hemocyanin is actually a copper porphyrin. Unfortunately hemocyanin and its derivatives do not have the intense and well defined spectra of the heme pigments. It is probably for this reason that so little is known about them.

3. *The Hemoglobin Model.* If hemoglobin has lost some of its relative importance amongst the heme pigments, it has certainly gained in interest as a *type* of biological molecule worthy of the most detailed study. Heme itself cannot combine with oxygen loosely and so cannot act as an oxygen carrier. The biological fitness of hemoglobin is the result of radical modifications in the properties of heme resulting from its combination with globin (Anson and Mirsky, 1925a). It now looks as if all the heme pigments (perhaps enzymes, too) are built on the hemoglobin model. The heme pigments in nature all have in common similar iron-porphyrin nuclei. They all, however, have individual peculiarities due in part to the specific natures of the substances to

which they are joined. Certainly this arrangement is an economical means of utilizing the varied potentialities of a single kind of substance.

4. *Cytochrome.* a. *Distribution.* That cytochrome exists in almost all aerobic cells has already been mentioned. In general the four spectroscopic bands of the pigment are more intense the more intense the metabolism of the tissue (MacMunn, 1886; and especially Keilin, 1925). The spectrum of cytochrome is, for instance, very intense and easy to observe in the extremely active wing muscles of bees which can contract several hundred times a second. Exact measurements of the concentration of cytochrome have, however, not been made. It is not even known whether the apparent intensities of the bands are a reliable measure of the concentration of the pigment.

b. *Oxidation and reduction.* Cytochrome can be oxidized and reduced by suitable chemical agents. The reduced form has the four bands while the spectrum of the oxidized form is practically invisible. Oxidation and reduction can also take place in the living cell even without the addition of oxidizing and reducing agents (MacMunn, 1886). Yeast and a transparent moth show the phenomena most readily (Keilin, 1925). If the yeast is well aerated its cytochrome is oxidized. If the yeast is exposed to a nitrogen-free atmosphere its cytochrome is reduced. In the case of the moth when the insect is active the cytochrome is partially oxidized. When the insect is quiet, its cytochrome becomes reduced again. Narcotics which influence the oxidation and reduction systems of cells naturally influence the oxidation and reduction of cytochrome (Keilin, 1925). In the presence of indifferent narcotics such as the urethanes, for example, cytochrome remains reduced. Apparently the urethanes inhibit the reducing system of the cell. In the presence of small concentrations of the specific inhibitors of cellular oxidations, cytochrome remains reduced even if the tissue is well aerated. Cytochrome is apparently not autoxidizable. Its oxidation is catalyzed by the cyanide sensitive oxidation ferments. The effect of cyanide may be very nicely observed spectroscopically in the transparent moth. When the insect is inactivated by cyanide vapors the cytochrome in the wing muscles becomes reduced. As the insect recovers after removal of the cyanide vapors, the cytochrome too recovers and may be oxidized once more.

c. *Composition.* MacMunn (1886) realized that cytochrome is related to hemochromogen. He knew that when cytochrome is oxidized its sharp bands practically disappear, just as is the case with hemochromogen. And by the action of alkali he was able to obtain from cyto-

chrome a typical hemochromogen. Nevertheless, MacMunn was not able to explain the exact relation of cytochrome to hemochromogen, nor to account for the fact that cytochrome has four bands while hemochromogen has two.

Keilin (1925) has analyzed the matter further. He has shown that cytochrome is not a single four-banded pigment but a mixture of three different hemochromogens. Three of the four bands are the three α bands of the three hemochromogens. The fourth band, the one farthest towards the blue, is the result of the fusion of the β bands of the three hemochromogens. Keilin's reasons for believing that cytochrome is a mixture, not a single pigment are these: 1. The relative intensities of the bands vary from the cytochrome of one tissue to that of another. This could not be true of a single pigment. It could easily be true of a mixture, the relative concentrations of the components of which are variable. 2. When cytochrome is oxidized and reduced the four bands do not disappear and reappear simultaneously. 3. Water extracts from yeast a single hemochromogen with an α band corresponding to one of the bands of cytochrome. 4. Cytochrome in general cannot be extracted as such. The complex nature of cytochrome has also been shown by a study of its reactions with pyridine and ammonia (Anson and Mirsky, 1925b).

None of the components of cytochrome react with CO in neutral solution, although they do combine with CO in strongly alkaline solution. Two, surely, of the components of cytochrome are not autoxidizable since in the presence of cyanide they are not oxidized by oxygen (Keilin, 1925).

The precise nature of the iron-porphyrin nuclei of the three components of cytochrome has not yet been decided although, as has already been stated, there is some evidence that there are two different hemes in cytochrome, one similar to the heme of hemoglobin, the other similar to the heme of chlorocruorin. Several porphyrins (Fisher and Fink, 1924; Fisher and Schneller, 1923; Schumm, 1925, 1926a, 1926b, 1926c) have been obtained from yeast. If these could be shown to have their origin in definite components of cytochrome then, of course, the nature of these porphyrins would provide valuable evidence concerning the nature of the hemes of cytochrome.

Keilin (1926) has succeeded in producing from ordinary pyridine hemochromogen a pigment or mixture of pigments with a spectrum similar to that of cytochrome. He believes his procedure to consist partly in the modification of the porphyrin by oxidation and reduction and partly in the precipitation of the pigment.

5. *Free Hemochromogen and Heme.* Some tissues which do not contain cytochrome in visible amount show a simple two-banded hemochromogen (MacMunn, 1886: Keilin, 1925). Many tissues, including those which do contain cytochrome, contain a free heme which is not visible but which can be made visible by suitable means (Keilin, 1926).

6. *Heme and Tissue Iron.* It has long been known that iron exists in tissues generally not as free iron but in some unidentified complex form. Since there are hemes present generally, some, at least, of this iron must be joined to porphyrin. There is evidence, not by any means conclusive, that about a third of the iron of baker's yeast and the wing muscles of bees is in the form of the same heme as that in hemoglobin (Anson and Mirsky, 1929a). The estimates of heme as an extremely small fraction of the total tissue iron (Von Euler, Fink and Hellstrom, 1927; Treibs, 1927) are definitely due to a technical error (Anson and Mirsky, 1929a).

IV. THE HEME PIGMENTS AND RESPIRATION. 1. *The Inhibitor Technique.* a. *The old heavy metal theory.* Substances which are stable when exposed to oxygen outside of the organism, within the organism are burned by oxygen readily. There must therefore be catalysts in the organism which speed up oxidations. Perhaps the greatest success so far attained in the study of biological oxidations has been the discovery of the nature of one of these catalysts. It has long been believed that an essential respiratory catalyst is a heavy metal compound, probably an iron compound, since iron is the heavy metal present in tissues in the highest concentration (Warburg, 1928). This conclusion rested on the fact that on the one hand respiration can be inhibited reversibly by minute concentrations of HCN or H_2S (Negelein, 1924), while on the other hand, heavy metal compounds can catalyze *in vitro* the oxidations of biological substances, but become catalytically inactive when combined with CN and H_2S. The inhibitor technique with HCN and H_2S had two great virtues. It permitted the study of the catalysis of respiration without any irreversible injury, serious disturbance, or permanent change in the tissue which was being studied. It resulted in the discovery that a catalyst essential to normal respiration has some of the characteristic properties of the heavy metal compounds. In fixing, however, the precise nature of the heavy metal compound acting as a respiratory enzyme, the old inhibitor technique and, in general, the experiments based on the old heavy metal theory were a failure. Warburg's original evidence that the heavy metal involved is iron was, in our opinion, completely unconvincing. And even if the respiratory ferment were an iron compound, the old experiments could not decide

what sort of an iron compound—an important matter since the properties of an iron compound depend to a large extent on the nature of the complex of which the iron is a part. As a result of the apparently inherent limitations of the inhibitor technique in its old form, progress in the search for the nature of the heavy metal respiratory catalyst simply stopped.

b. *The new methods.* The new investigations of Warburg (Warburg and Negelein 1929d), however, have revolutionized the study of biological oxidations. The old inhibitor technique with its great and unique virtues of not involving any permanent injury to the cell and permitting the study of normal respiration has been retained. But to it there has been added the new virtue of specificity. And as a result it has been demonstrated that an essential respiratory enzyme is not only a heavy metal compound but in particular a heme compound. This great and sudden change has been brought about because, following the newer study of the heme pigments in nature and *in vitro*, the inhibition of respiration has been taken up from the point of view of the particular properties of the heme pigments rather than from the point of view of the general properties of heavy metal compounds and because Warburg has added to the old purely qualitative inhibitor technique an entirely new, quantitative and powerful kind of spectrum analysis.

c. *Inhibition by CO.* The new qualitative facts are these. The respiration of tissues may be inhibited by CO in the dark completely and reversibly. By the action of visible light this inhibition may be abolished. Iron-porphyrin compounds *in vitro* like the respiratory ferment *in vivo* can combine with CO reversibly to form CO derivatives which may be dissociated by visible light. These iron-porphyrin compounds can catalyze oxidations. Their CO derivatives are catalytically inactive. Outside of the iron-porphyrins no other substances have as yet been shown to react reversibly with CO to form light-sensitive derivatives. It still remains possible, however, that some other kind of iron compound as yet unknown or unstudied reacts with CO in just the same manner as the iron-porphyrin compounds or the respiratory ferment, and that the respiratory ferment is this other kind of compound. This uncertainty cannot be avoided in the qualitative form of the inhibitor technique.

d. *The new spectrum analysis.* So far the experiments with CO have been exactly the same as those with CN, the only advantage of CO being that it is a more specific although still not an absolutely specific inhibitor. The part of Warburg's present procedure which yields an entirely new

kind of information is the spectrum analysis which permits the demonstration not only that the respiratory ferment has some peculiar properties also possessed by iron-porphyrin compounds but that the respiratory ferment definitely *is* an iron-porphyrin compound. Light of one color is more effective in influencing the CO inhibition of respiration than light of another color. This means that the CO compound of the respiratory ferment is a pigment which absorbs one kind of light more than another. Suitable quantitative experiments on the effectiveness of different kinds of light permit the calculation of the precise extent to which the CO compound of the respiratory ferment absorbs different kinds of light, in other words, the calculation of the ferment's spectrum. The spectrum found is that of a heme compound. No substance, regardless of how it can react with CO or any other inhibitor, can have this spectrum unless it has an iron-porphyrin molecular structure.

2. *The Respiratory Ferment.* a. *Reactions with CO, O_2 and CN.* It has long been known that small concentrations of CO poison vertebrates. Claude Bernard (1883) discovered that CO acts by combining with hemoglobin and thus rendering the red blood corpuscles unfit to transport their quota of oxygen. Animals which do not depend on hemoglobin for the transportation of oxygen are not influenced by *low* concentrations of CO. Claude Bernard (1883) himself, however, stated that one-sixth of an atmosphere of CO prevents the germination of seeds. This experiment has not been confirmed but it has been shown (Linossier, 1889; Wehmer, 1925) that concentrations of CO as high as four-fifths of an atmosphere do have a poisonous effect on seeds and seedlings. This general poisonous effect of CO was independently rediscovered by Warburg (1926; see also Haldane, 1927) who showed further that the inhibitory effect is on respiration, not on anaerobic fermentation (Warburg, 1927), that it is reversible, and that it may be abolished by light.

Of the CO heavy metal compounds only the CO iron compounds are sensitive to light (iron pentacarbonyl, Mond and Langer, 1891; CO hemoglobin, Haldane and Smith, 1896; CO hemochromogen, Anson and Mirsky, 1925a; Krebs, 1928; CO ferrocysteine, Cremer 1928, 1929; CO carbylamine hemoglobin, Warburg, Negelein and Christian 1929). And of the iron compounds only some iron porphyrins have an affinity for CO comparable with that of the respiratory ferment and the CO compounds of the iron porphyrins alone have a sensitivity to light comparable with that of the CO compound of the respiratory ferment. Furthermore, it has not yet been demonstrated that any iron compound

other than an iron porphyrin can combine reversibly with CO in the dark, as the respiratory ferment does. So from the reactions of the respiratory ferment with CO it is probable that the respiration ferment is a heme derivative—a conclusion which in any case is proven by the fact that the spectrum of the CO compound of the respiratory ferment is a heme spectrum.

Since the known heme compounds have a great affinity for CO it seems surprising that so high a concentration of CO is needed to inhibit respiration or the heme catalysis of the oxidation of cysteine. The reason for this is that these inhibitions depend not only on the CO pressure but, unlike normal uninhibited respiration, on the O_2 pressure also (Warburg, 1926, 1927; Warburg and Negelein, 1928a).

The ferment presumably acts as a catalyst by being oxidized by oxygen and then reduced by substances in the cell the oxidation of which in the procedure constitutes respiration. On this basis the rate of respiration depends on the concentration of the oxidized ferment. In the absence of CO the ferment is practically completely oxidized even when the oxygen pressure is low, so that increasing the O_2 pressure cannot increase the concentration of the oxidized ferment and hence the rate of respiration. In the presence of CO, CO and O_2 compete for the reduced ferment and so the concentration of the oxidized ferment, and hence the rate of respiration, depends on the CO/O_2 ratio according to the equation

$$\frac{[\text{Oxidized ferment}]}{[\text{CO ferment}]} \times \frac{[CO]}{[O_2]} = K = 10$$

Since the ferment has such a great tendency to become oxidized, the fact that CO can inhibit respiration at all indicates that the respiratory ferment like other heme compounds has a high affinity for CO.

It is a familiar fact (Douglas, Haldane, and Haldane, 1912) that hemoglobin when exposed to a mixture of CO and O_2 reacts reversibly with both these gases according to the mass law equation

$$\frac{[HbO_2]\,[CO]}{[HbCO]\,[O_2]} = K = \frac{1}{100}$$

K here is a true equilibrium constant. Its value means that the affinity of hemoglobin for CO is 100 times as great as the affinity for O_2. It might be supposed from the similarity of the two equations that the respiratory ferment reacts with O_2 and CO exactly as does hemoglobin,

the only difference being that the ferment has a relatively greater affinity for O_2 than for CO. This is definitely not the case. For while oxyhemoglobin dissociates spontaneously, this being thermodynamically the reverse of the oxygenation of hemoglobin, the oxidized respiratory ferment is constantly being directly reduced by the oxidizable substances of the cell just as in the heme catalysis of the oxidation of cysteine, the oxidized heme is constantly being reduced by the cysteine which is thus oxidized. The oxidation and reduction of the respiratory ferment are then two different reactions, not simply the same reaction in two opposite directions as in the case of hemoglobin. In addition to direct reduction of the respiratory ferment there may also be spontaneous dissociation, as Warburg supposes. Such spontaneous dissociation of molecular oxygen would not be of use in catalyzing oxidations. There is, then, not equilibrium in the cell but a steady state in which the ferment is being oxidized by oxygen as fast as it is being reduced. Other things being equal this steady state depends on the concentration of substances which can be oxidized by the ferment. Experimentally, therefore, the K of the ferment is *not* the ratio of two affinity constants but a distribution constant which states what fraction of the ferment in a given steady state is in the oxidized, catalytically active form and what fraction in the carbon monoxide, catalytically inactive form.

It cannot be determined from the mere existence of a distribution constant how the ferment is oxidized and reduced, what the nature and composition of the oxide is, or whether it is analogous to oxyhemoglobin or methemoglobin. The symbol FeO_2 used by Warburg to denote the oxidized ferment is purely arbitrary and should not be taken to mean that the ferment has been proven to have the composition FeO_2.

The inhibition of respiration by CN is not influenced by the O_2 pressure; which indicates that CN combines with the already oxidized ferment and so does not compete with O_2 for the reduced ferment (Warburg, 1927). In harmony with this is the fact that in *neutral* solution CN has a great affinity for oxidized but not for reduced heme compounds (Anson and Mirsky, unpublished experiments). It is not yet proven beyond doubt that CN combines with the same substance in the cell as does CO.

Heme can catalyze the oxidation of many substances (Robinson, 1924; Harrison, 1924; Krebs, 1928, 1929; Kuhn and Meyer, 1928, 1929). Most of these catalyses are inhibited by CN but not the oxidation of olive oil (Kuhn and Meyer, 1929). Similarly the catalysis of the oxidation of linseed oil by hemoglobin is not sensitive to CN (Robinson, 1924).

It is also known that the respiration of the plant Chlorella is not inhibited by CN unless glucose is added (Warburg 1919; Emerson, 1927; Genevois, 1927a, 1927b). And it has been claimed that although the respiration of yeast and other cells is completely inhibited by CN that of some tissues is inhibited by CN to only sixty per cent. The remaining respiration has a respiratory quotient of 0.6 instead of the normal 0.85 (Dixon and Elliott, 1929). It has been suggested (Kuhn and Meyer, 1929) that all these facts fit in with the hypothesis that the oxidation of carbohydrate but not of fat is inhibited by CN and that the uninhibited metabolism of Chlorella and some animal tissues is a fat metabolism. Perhaps the oxidized heme ferment even when combined with CN can still oxidize fat. It is clearly desirable that the supposed cases of the incomplete inhibition of respiration by CN be confirmed and that the effect of CO on the respiration of Chlorella be determined.

Although the oxidation of substances like linseed oil is not inhibited by CN when the catalyst is heme or methemoglobin, when the catalyst is inorganic iron, CN produces a complete inhibition of the oxidation. This is a striking example of the differences in the properties of iron which result when the iron becomes a part of an organic complex. On the basis of the old iron theory, the fact that a catalysis of oxidation is not inhibited by CN would incorrectly be taken as proof that the catalysis was not an iron catalysis.

It has long been known that many tissues give a blue color with the Nadi reagent, which is the so-called indophenol oxidase reaction. This oxidase reaction is inhibited by CO and CN exactly as is normal respiration, so that the heme ferment of normal respiration is probably also involved in the artificial oxidase system (Keilin, 1929). According to Harrison (1929) the ferment produces hydrogen peroxide and this peroxide plus peroxidase are what give the indophenol reaction.

b. *The significance of spectra.* The spectrum of a substance is an expression of the degree of absorption of light of every wave length. This information is best given in the form of a curve in which absorption is plotted against wave length (fig. 1). Usually only the *shape* of the curve is measured, that is, the *relative* extents to which different kinds of light are absorbed. A complete description of the spectrum, however, demands also a statement of the *absolute* amount of light absorbed per molecule of pigment. Pigments of similar chemical structure yield spectrum curves of the same general shape and of similar absolute dimensions. If two pigments have identically the same spectrum then they are identical substances. Substances may thus be characterized

or identified by their spectra just as accurately and certainly as by their chemical compositions or melting points. This optical method has the great advantage that it does not necessarily involve the isolation and purification of the substance.

c. *Theory of the new spectrum analysis.* (Warburg, 1927; Warburg and Negelein, 1928d; Warburg, Negelein and Christian, 1929.) In general the amount of light absorbed by a solution of a pigment may be

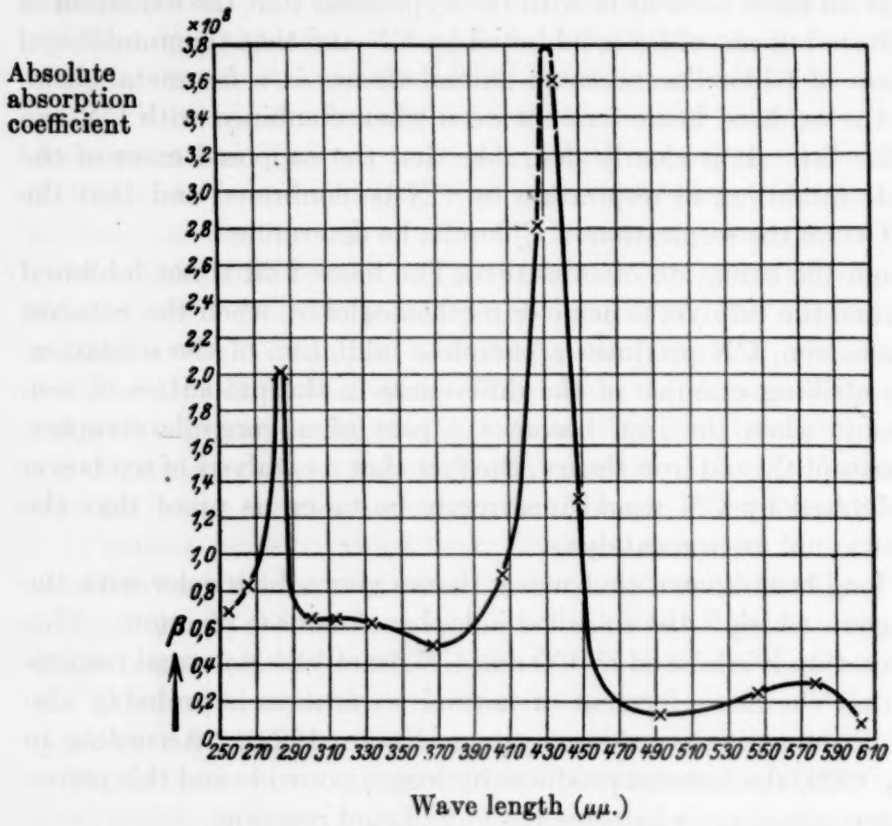


Fig. 1. Absorption spectrum of the respiration ferment

estimated in two different ways. The intensity of the light before and after it has passed through the solution may be measured directly with suitable physical instruments. Or the extent of some specific chemical change in the pigment resulting from the absorption of light may be used as a measure of the amount of light which has been absorbed. The direct physical method requires a solution of pigment free of other pigments and concentrated enough to have a measurable absorption. Clearly the physical method is out of the question for a ferment still present in the cell in minute concentration together with many other

substances which absorb light. If the spectrum of the respiratory ferment is to be found it must be by the indirect chemical method, by the use of the only known specific chemical effect of light on the respiratory ferment, namely, its effect in dissociating the compound of the ferment with CO. The amount of CO which reacts with the respiratory ferment is too small even to be detected. What can, however, be determined experimentally is the rate of respiration. If one assumes that the rate of respiration is directly proportional to the concentration of ferment which is not combined with CO, then the effect of light in increasing the rate of the inhibited respiration is a direct and magnified measure of the CO split off by the light. Light absorbed by other cellular constituents does not influence respiration by splitting off CO from the respiratory ferment and so is not measured. In the absence of CO light has no effect on respiration.

What must be known now is the relation between the rate at which CO groups are split off by a given kind of light, determined by measurements of respiration, and the extent to which the pigment absorbs light; in other words, its spectrum. It may be seen at once that the more strongly the CO pigment absorbs light, the more rapidly will CO groups be split off as a result of the absorption of light. More precisely, the rate at which CO groups are split off is equal to the rate at which light is absorbed times the number of CO groups split off by a unit amount of light. The desired absolute absorption coefficient, β, defines the rate of light absorption by each molecule of the pigment, FeCO, when the light intensity, i, is unity. The rate of absorption of light per unit volume is therefore $i\beta$ [FeCO]. To know how much light it takes to split off one CO group and how this value varies with the frequency of the light, one must make the simplest assumption, that the reaction obeys Einstein's law of photochemical equivalence. This law states that regardless of the frequency of the light, for every *quantum* of light absorbed a definite constant number, E, of CO groups is split off. The rate at which CO groups are split off, $\frac{d[FeCO]}{dt}$, is therefore equal to the number of groups split off per quantum of light absorbed, E, times the rate at which quanta are absorbed. This rate is equal to the rate at which light energy is absorbed, $i\beta$[FeCO], divided by the amount of energy in one light quantum ($h\nu$, h a constant, ν the frequency of the light). So

$$d\,[FeCO]/dt = E\,\frac{i}{h\nu}\,\beta\,[FeCO]$$

If i is kept sensibly constant, that is to say, if only a small part of the light is absorbed, then $E\frac{i}{h\nu}\beta$ is a constant, V, and

$$\frac{d\,[FeCO]}{dt} = V\,[FeCO] \qquad V = E\,\frac{i}{h\nu}\,\beta$$

V is simply the velocity constant of the reaction. For a different light intensity and frequency, we have

$$V' = E'\,\frac{i'}{h\nu'}\,\beta'$$

and

$$\frac{V}{V'} = \frac{i}{i'}\,\frac{\nu'}{\nu}\,\frac{\beta}{\beta'}$$

In order, therefore, to learn the absolute absorption coefficient, β, it is necessary to know the absolute value of E and the V for a given intensity and frequency of light. The measurement of both these values is difficult experimentally. But in order to obtain simply the shape of the absorption curve, that is to say, the relative extents to which different kinds of light are absorbed $\frac{\beta}{\beta'}$, it is necessary to measure only the ratio of two velocity constants $\frac{V}{V'}$, which is relatively easy, and to know that E is some constant, that Einstein's law is obeyed.

d. *The relative spectrum* (Warburg, 1927; Warburg and Negelein, 1928b, 1929b). $\frac{V}{V'}$, and hence $\frac{\beta}{\beta'}$ may be calculated from the effect of light in increasing the constant respiration in the presence of CO, that is, from the effect of light on the distribution constant K:

$$K = \frac{[\text{Oxidized Ferment}]}{[\text{CO Ferment}]}\,\frac{[CO]}{[O_2]}$$

$$= \frac{[\text{Oxidized Ferment}]}{[\text{Total Ferment}] - [\text{Oxidized Ferment}]}\,\frac{[CO]}{[O_2]}$$

The concentration of the oxidized ferment under any conditions is proportional to the rate of respiration. Since in the absence of CO all the

ferment is oxidized, respiration in the absence of CO is a measure of the total concentration of ferment. So K is obtained by measuring the respiration both in the presence and the absence of CO. From the K's for two different kinds of light it is possible to calculate $\frac{V}{V'}$, for the rate of respiration and hence K depends on how much of the ferment is combined with CO. Other things being equal this depends on the ratio of the velocity constant of dissociation of the CO ferment compound to the velocity constant of combination of ferment with CO. In the dark there is only spontaneous dissociation so that the ratio is $\frac{V_{\text{spontaneous dissociation}}}{V_{\text{combination}}}$. In the light there is both spontaneous dissociation and dissociation due to the absorption of light so that the ratio becomes $\frac{V_{\text{spontaneous dissociation}} + V_{\text{light dissociation}}}{V_{\text{combination}}}$. From these two ratios one can calculate $\frac{V_{\text{light dissociation}}}{V_{\text{combination}}}$. If light of another wave length is used one obtains $\frac{V'_{\text{light dissociation}}}{V_{\text{combination}}}$. The velocity constant of combination remains the same since it is not affected by light. By elimination of the combination constant one finally obtains $\frac{V}{V'}$ and hence the desired $\frac{\beta}{\beta'}$.

It is impossible to calculate an individual absolute velocity V, and hence the absolute absorption coefficient, β, from K because K represents a steady state. A steady state is the result of a balance of reactions. An infinite number of combinations of individual velocities could give the same balance and hence the same K.

The relative spectrum of the respiratory ferment obtained in the way just described has the characteristic bands of CO heme compounds. The same spectrum is obtained for different tissues, for different substances being burned, and for different extents of inhibition by CO and about the same degree of inhibition is obtained in a given pressure of carbon monoxide under these different conditions (Warburg, 1929a; Warburg and Negelein, 1929c). Such reasonable and consistent results could hardly be obtained were the method and the assumptions underlying it seriously incorrrect.

It is remarkable that when the ferment system of respiration is extracted from the cell or at least when the attempt is made to extract it, the catalytic system in the extract seems more complicated rather than

more simple than it is in the cell. The rate of oxidation in the extract and the sensitivity of the catalysis to inhibition are variable; they change with time and with the nature of the substance being burned. From the fact, therefore, that many so-called oxidases may be obtained from cells by different procedures, one cannot conclude that this great variety of oxidases actually exists in the normal cell. It is indeed of great advantage to the cell to have the oxidation of many metabolites catalyzed by the same ferment (Warburg, 1929a).

Further evidence in favor of the soundness of the procedure for obtaining the relative spectrum is given by *in vitro* experiments with known substances. The oxidation of cysteine to cystine is catalyzed by nicotine hemochromogen and this catalysis is inhibited by carbon monoxide (Krebs, 1928). The spectrum of nicotine hemochromogen may therefore be learned in precisely the same manner as was the spectrum of the respiratory ferment by studying the effect of light on the CO inhibition of the hemochromogen catalysis of the oxidation of cysteine. In this case, however, the spectrum may also be ascertained by direct physical measurements of the absorption of light by solutions of nicotine hemochromogen. There is complete agreement in the spectra obtained by the two independent methods (Warburg and Negelein, 1928c). This agreement justifies the two assumptions underlying the method, that the catalysis is proportional to the concentration of oxidized heme and that Einstein's law is obeyed.

e. *The absolute spectrum* (Warburg, 1928d, 1929a; Warburg and Negelein, 1929b). It has been shown that β, the absolute absorption coefficient, is related to the velocity with which CO groups are split off from combination with a pigment because the more the pigment absorbs light, the faster will CO groups be split off as a result of this absorption. In the form of equations:

$$\frac{d\,[FeCO]}{dt} = \frac{E\,i\,\beta}{h\nu}\,[FeCO]$$

$$= V\,[FeCO]$$

$$V = E\,\frac{i}{h\nu}\,\beta$$

$\frac{i}{h\nu}$ may always be measured by direct physical methods. In the case of known solutions of FeCO pigments, β may also be measured by direct

physical methods. V may be obtained from the rate at which the pressure in a closed system changes as CO molecules are liberated. In this way E, the number of CO groups split off when one quantum of light is absorbed may be calculated. For a variety of FeCO compounds, iron pentacarbonyl (see also Eyber, 1929) CO ferrocysteine and CO hemochromogen, it is found that a single molecule of CO is liberated for each quantum of light absorbed. It takes the absorption of four quanta, however, to cause the dissociation of one molecule of CO from CO carbylamine hemoglobin. (Warburg, Negelein and Christian, 1929.) Once E is known the β of the respiratory ferment may be obtained from V. In other words, V and $\frac{i}{h\nu}$ are measured and they give the product E β. In the case of known pigments E is obtained from β. In the case of the respiratory ferment β is then obtained from E.

Perhaps the most brilliant part of Warburg's investigations consists in the overcoming of the serious difficulties involved in the measurement of V for the respiratory ferment. It is not feasible to measure accurately the rate at which respiration changes as CO groups are split off when the carbon monoxide derivative of the ferment is exposed to a definite intensity of light. But the difficult problem of measuring the changing respiration of yeast in a constant light has been reduced to the simpler problem of measuring the constant respiration of yeast in a changing light. When yeast whose respiration is inhibited by CO is exposed to light the rate of respiration rises until it reaches the value of the constant respiration in light. When the light is turned off the rate of respiration drops until it reaches the value of the constant respiration in the dark. The total respiration of the yeast in the whole period of oscillation of the light clearly depends on the values of the constant respiration in the dark and in the light (both of which can be measured separately) and on the absolute rates at which the CO groups combine with and become detached from the ferment molecule. Other things being equal the total respiration in a period of oscillation depends on V. The remarkable fact which has been demonstrated theoretically and experimentally is that when the light is made to oscillate faster and faster, a point is reached where the respiration is sensibly constant and not influenced by further increases in the rate of oscillation, and yet is still a function of V. It is from the value of this constant respiration in rapidly oscillating light that V and hence β are obtained.

f. *Peculiarities of the spectrum.* Recently the ultra-violet spectrum of the respiratory ferment has been measured (Warburg and Negelein,

1929b). The striking fact appeared that the respiratory ferment has a marked band in the ultra-violet at about 2700 Å which is lacking in all the pure heme compounds. Proteins, however, due to their tryptophane groups have a characteristic band in the ultra-violet with a wave length of maximum absorption corresponding to that of the respiratory ferment. Although the absolute absorption coefficients of proteins at 2700 Å vary with their tryptophane contents their order of magnitude is the same as that of the respiratory ferment. The simplest conclusion is that the heme of the respiratory ferment is joined to some nitrogenous group probably to a protein, that the respiratory ferment is a hemochromogen. Unfortunately many substances have bands in the ultra-violet, so that the hemochromogen nature of the respiratory ferment may not yet be taken as proven by its spectrum.

It follows from the fact that light absorbed by the non-heme part of the ferment influences the reaction with CO by which the spectrum is measured, that this non-heme part influences the reactivity of the ferment iron, and probably its catalytic reactivity, just as globin influences the properties of hemoglobin's iron (Anson and Mirsky, 1925a). In harmony with this view are the experiments which show that heme only when joined to a hemochromogen-forming nitrogen group is a very effective catalyst of the oxidation of cysteine and that the CO compound only of the hemochromogen is very sensitive to light. Heme itself not combined with a nitrogen group is only a feeble catalyst and the CO compound of free heme has only a small sensitivity to light (Krebs, 1928).

Although the visible, unlike the ultra-violet spectrum of the respiratory ferment, does not show any striking difference from the spectrum of ordinary CO reduced heme prepared from hemoglobin, still the bands are definitely more towards the red end of the spectrum than those of ordinary CO reduced heme (Warburg and Negelein, 1928d). They are much closer to the bands of CO chlorocruorin (Warburg, 1929c). The significance of this shift to the red is not known. It may be due to a partial precipitation of the pigment, to a difference in the heme similar to that of chlorocruorin, to the influence of a peculiar nitrogenous substance, or perhaps to a yet undiscovered inaccuracy of the technique.

g. *The concentration and effectiveness of the respiratory ferment.* The reactions of the respiratory ferment with CO and O_2 permit not only an exact knowledge of the ferment's spectrum but also a rough estimate of the ferment's concentration. A maximum value to the amount of ferment iron is fixed by the amount of CO absorbed in the complete

inhibition of respiration. This is so small that it cannot be measured at present. The sensitivity of the procedure is such that this means that in the case of baker's yeast the ferment iron cannot be more than 4×10^{-7} grams per gram of yeast, which is about one two hundred fiftieth the total iron (Warburg and Kubowitz, 1928) and a small part even of the total heme iron. A minimum value of roughly 3×10^{-8} grams iron per gram of yeast can be calculated if one assumes that every collision between a ferment iron atom and an oxygen molecule results in a combination and in the consumption of one molecule of oxygen in respiration (Warburg, 1929b). The minimum and maximum values are both of the same extremely low order of magnitude.

From the approximate value of the concentration of the ferment iron it follows that one atom of ferment iron "activates" very roughly ten thousand molecules of oxygen per minute. To do this the ferment iron must react with oxygen very rapidly. Independent evidence of such a rapid reaction is provided by the fact that the rate of normal respiration is the same in pure oxygen as in pressures of oxygen as low as 10^{-5} atmospheres (Warburg, 1929b). The ferment iron reacts so rapidly with oxygen that it is kept oxidized even when the pressure of oxygen is low so that raising the pressure can have no further effect. The only reason the O_2 pressure is important in CO inhibition is, as has already been stated, because the CO competes with the O_2 for the reduced iron.

h. *Relation to cytochrome.* The respiratory heme is present in too small a concentration to permit of direct spectroscopic detection. This means that the ferment is not identical with the heme pigments which have actually been observed. In any case the components of cytochrome are excluded for under normal conditions they do not combine with CO. It is a curious fact that the iron which has finally been proven to be concerned in biological respiration is not the iron which has always been estimated or the iron which has always been seen.

3. *The Function of Cytochrome.* The question naturally arises what the function of cytochrome is. Experimentally nothing is known. Some properties of cytochrome are known qualitatively; it is known for instance that it can be oxidized and reduced. But what cytochrome actually does accomplish in normal respiration still remains to be demonstrated. Several theoretical possibilities have been suggested (Keilin, 1929; Dixon and Elliott, 1929). It is of course conceivable that the ferment which combines with CO is only one catalyst in a complicated catalytic system of which cytochrome and glutathione are essential

parts. There is, however, as yet no adequate experimental basis for a discussion of the question.

The heme pigments have been given credit for the peroxidase action of cells (Keilin, 1929) on the grounds that all hemes are to some extent peroxidases and that the peroxidase action of tissues is in a general way parallel to their content of visible heme pigments. There are, however, so many substances whose concentrations go with the intensity of tissue metabolism that parallelism of concentration is hardly sufficient evidence of identity of structure.

It has also been claimed (Keilin, 1925, 1929) that cytochrome is a respiratory pigment because it can be oxidized and reduced. The chromotologists have always called every pigment which can be oxidized or reduced a respiratory pigment—why, it is not clear. There are many colorless substances in the cell which can be and probably are oxidized and reduced. This property is not taken as proof that they are respiratory substances.

CONCLUSION

It is clear from the investigations which have been discussed in this review that apart from the familiar hemoglobin and chlorophyll, there are many other porphyrin compounds in nature, in all plants and animals. What the functions of these pigments are, is with one exception, unknown, despite the fact that some of them are present in such concentration that their spectra may be observed directly. The one pigment whose function is known is an iron porphyrin, a heme compound which is an essential catalyst of respiration. It is remarkable that even the existence of this heme has been discovered, no less its function. For the heme ferment of respiration is present in only one part in a hundred million. Isolation of such a minute amount of ferment seems out of the question. And the ordinary methods would not permit of the identification of the nature of such a small amount of substance even if it could be isolated. Fortunately the actual measurement of the ferment's spectrum by the carbon monoxide inhibitor technique depends on the results of the presence of the ferment which are great despite the ferment's small concentration. The demonstration of the heme nature of the respiratory ferment is all the more striking when one considers that on the one hand, the chemical nature of no other intracellular enzyme present in minute amount is known, and that, on the other hand, extremely little is known about the normal function of even the ordinary, well studied constituents of the cell which are present in large amounts.

The universal distribution of the porphyrin pigments and the heme nature of the respiratory ferment have been discovered primarily because hemoglobin and its derivatives have been so readily available for study and because these substances have such well.defined spectroscopic properties. Once, for example, the reactions and spectra of the hemoglobin derivatives were known, there was no difficulty in identifying cytochrome as a heme compound. More particularly, once the characteristics of the hemochromogens were understood, it became possible to show that the cytochrome complex is a mixture of hemochromogens. Similarly, Warburg's beautiful method for obtaining the spectrum of the respiratory ferment depends on a knowledge of the reactions of the heme pigments with carbon monoxide and of the spectra of these carbon monoxide compounds.

A great deal remains to be found out about the heme pigments. One wonders what the physiological significance is of the close chemical relationship between chlorophyll and heme. Nothing, moreover, is known about the function of cytochrome. And relatively little is known about the effect on the catalytic activity of heme of modification of the porphyrin nucleus and of combination of heme with various nitrogenous substances. It is to be hoped, finally, that the inhibitor technique in the new photochemical form which was devised as a result of the peculiar properties of the heme ferment of respiration will now make possible the discovery of the spectra of other enzymes.

BIBLIOGRAPHY

ANSON M. L. 1926. Thesis, Cambridge University

ANSON, M. L. AND A. E. MIRSKY. 1925a. Journ. Physiol., lx, 50.

1925b. Journ. Physiol., lx, 161.

1925c. Journ. Physiol., lx, 221.

1925d. Journ. Gen. Physiol., ix, 169.

1928. Journ. Gen. Physiol., xii, 273.

1929a. Journ. Gen. Physiol., xii, 401.

1929b. Journ. Gen. Physiol., xiii, 121, 133.

1930a. Journ. Gen. Physiol., xiii, 469.

1930b. Journ. Gen. Physiol., in the press.

ARNOLD, V. 1900. Zeitschr. Physiol. Chem., xxix, 78.

BERNARD, C. 1883. Leçons sur les Effets des Substances Toxiques, Paris.

BERTIN-SANS, H. ET J. MOITESSIER. 1892. C. R. Acad. Sci., cxiv, 923.

1893a. C. R. Acad. Sci., cxvi, 401.

1893b. Bull. Soc. Chim., iii Serie., ix, 243.

1893c. Bull. Soc. Chim., iii Serie., ix, 380.

BERZELIUS, J. 1840. Lehrbuch der Chemie, Dresden und Leipzig, ix, 60.

BIERICH, R., A. ROSENBOHM, UND K. KALLE. 1927. Zeitschr. Physiol. Chem., clxiv, 207.
CONANT, J. B. 1923. Journ. Biol. Chem., lvii, 401.
CONANT, J. B. AND C. O. TONGBERG. 1930. Journ. Biol. Chem., lxxxvi, 733.
CREMER, W. 1928. Biochem. Zeitschr., cxciv, 231.
1929. Biochem. Zeitschr., ccvi, 228.
DHÉRÉ, C. ET C. BAUMELER. 1926. C. R. Soc. Biol., xcv, 628.
DHÉRÉ, C. ET G. VEGEZZI. 1917a. Journ. Physiol. et Pathol. Génér., xvii, 44.
1917b. Journ. Physiol. et Pathol. Génér., xvii, 53.
1919. Journ. Physiol. et Pathol. Génér., xviii, 239.
DILLING, W. J. 1910. Atlas of crystals and spectrum of hemochromogen. Stuttgart.
DOUGLAS, C. G., J. S. HALDANE AND J. B. S. HALDANE. 1912. Journ. Physiol., xliv, 278.
DIXON, M. 1929. Biol. Rev. and Biol. Proc. Cambr. Phil. Soc., iv, 352.
DIXON M. AND K. ELLIOTT. 1929. Biochem. Journ., xxiii, 812.
DONOGANY, Z. 1893. Thierchemie, xxiii, 126.
EMERSON, R. 1927. Journ. Gen. Physiol., x, 469.
VON EULER, H., H. FINK, UND H. HELLSTROM. 1927. Zeitschr. Physiol. Chem., clxix, 10.
EYBER, G. 1929. Zeitschr. Physikal Chem., cxliv, 1.
FISCHER, H. 1924. Hdbch. der Biochemie (Oppenheimer) 2 Aufl. 1, 351.
1929a. Die Naturw., p. 611.
1929b. Ann. d. Chem., cdlxxi, 285.
FISCHER, H. UND H. FINK. 1924. Zeitschr. Physiol. Chem., cxl, 57.
FISCHER, H. UND HAHN. 1913. Ber. Chem. Ges., xlvi, 2308.
FISCHER, H. UND HILGER. 1923. Zeitschr. Physiol. Chem., cxxviii, 167.
FISCHER, H. UND F. KÖGL. 1923. Zeitschr. Physiol. Chem., cxxxi, 241.
FISCHER, H. UND H. SCHEYER. 1924. Ann. de. Chem., cdxxxix, 185.
FISCHER, H. UND K. SCHNELLER. 1923a. Zeitschr. Physiol. Chem., cxxviii, 230.
1923b. Zeitschr. Physiol. Chem., cxxxv, 252.
FISCHER, H. UND A. SCHORMÜLLER. 1929. Ann. d. Chem., cdlxxiii, 222.
FISCHER, H. UND F. SCHWERTEL. 1928. Zeitschr. Physiol. Chem., clxxv, 248.
FISCHER, H. UND ZEILE. 1929. Ann. d. Chem., cdlxviii, 98.
FOX, H. M. 1926a. Arch. d. Phys. Biol., v, 85.
1926b. Proc. Roy. Soc., B., xcix, 199.
GENEVOIS, L. 1927a. Biochem. Zeitschr., clxxxvi, 461.
1927b. Biochem. Zeitschr., cxci, 147.
GRIFFITHS, A. B. 1892a. C. R. Acad. Sci., cxiv, 1277.
1892b. Proc. Roy. Soc. Edin., xix, 117.
HALDANE, J. S. AND L. SMITH. 1896. Journ. Physiol., xx, 497.
HALDANE, J. B. S. 1927. Nature, cxix, 352.
HARRISON, D. C. 1924. Biochem. Journ., xviii, 1009.
1929. Biochem. Journ., xxiii, 982.
HARTRIDGE, H. 1912. Journ. Physiol., xliv, 1.
HASSELBALCH, K. A. 1909. Biochem. Zeitschr., xix, 430.
HAUROWITZ, F. UND H. WAELSCH. 1929. Zeitschr. Physiol. Chem., clxxxii, 82.
HILL, R. 1926. Proc. Roy. Soc. B., c, 419.
1929. Proc. Roy. Soc., B., cv, 112.

HILL, R. AND H. F. HOLDEN. 1926. Biochem. Journ., xx, 1326.
1927. Biochem. Journ., xxi, 625.
HOLDEN, H. F. AND M. FREEMAN. 1929. Australian Journ. Exper. Biol. and Med., vi, 79.
HOPPE-SEYLER, F. 1862. Virchow's Arch., xxiii, 446.
1870. Ber. Chem. Ges., iii, 229.
1878. Zeitschr. Physiol. Chem., ii, 154.
1879. Zeitschr. Physiol. Chem., iii, 339.
1880. Zeitschr. Physiol. Chem., iv, 193.
1881. Zeitschr. Physiol. Chem., v, 75.
1890a. Zeitschr. Physiol. Chem., xiv, 106.
1890b. Zeitschr. Physiol. Chem., xiv, 329.
HOPPE-SEYLER, F. AND THIERFELDER. 1893. Hdbch. d. phys. u. path.-chem. Analyse. Berlin, p. 214.
KEILIN, D. 1925. Proc. Roy. Soc. B., xcviii, 312.
1926. Proc. Roy. Soc., B., c, 129.
1927. C. R. Soc. Biol., Réunion Plenière, May 27.
1929. Proc. Roy. Soc. B., civ, 206.
VAN KLAVERN, K. H. L. 1901. Zeitschr. Physiol Chem., xxxiii, 293.
KREBS, H. A. 1928. Biochem. Zeitschr., cxciii, 347.
1929. Biochem. Zeitschr., cciv, 343.
KRUNKENBERG, C. 1880, 1882. Vergl. Studien.
KUHN, R. UND K. MEYER. 1928. Naturw., xvi, 1028.
1929. Zeitschr. Physiol. Chem., clxxxv, 193.
LAIDLAW, P. P. 1904. Journ. Physiol., xxxi, 464.
LANKESTER, E. R. 1867. Journ. Anat. and Physiol., ii, 114.
1870. Journ. Anat. and Physiol., iii, 119.
LEVY, L. 1889. Zeitschr. Physiol. Chem., xiii, 309.
LINOSSIER. 1889. C. R. Acad. Sci., cviii, 820.
MACMUNN, C. A. 1885a. Quart. Journ. Micr. Sci., xxv, 469.
1885b. Phil. Trans. Roy. Soc., dcxli.
1886. Phil. Trans. Roy. Soc., clxxvii, 267.
1887. Journ. Physiol., viii, 57.
1889a. Quart. Journ. Micr. Sci., xxx, 51.
1889b. Zeitschr. Physiol. Chem., xiii, 309.
1889c. Zeitschr. Physiol. Chem., xiii, 497.
1890. Zeitschr. Physiol. Chem., xiv, 328.
MENZIES, J. A. 1894. Journ. Physiol., xvii, 415.
1915. Journ. Physiol., xlix, 452.
MILNE-EDWARDS. H. 1838. Ann. Sci. Nat., x, 212.
MIRSKY, A. E. 1926. Thesis Cambridge University.
MIRSKY, A. E. AND M. L. ANSON. 1929a. Journ. Gen. Physiol. xii, 581.
1929b. Journ. Gen. Physiol. xiii, 133.
1930. Journ. Gen. Physiol. xiii, 477.
MOND, L. AND C. LANGER. 1891. Journ. Chem. Soc., lix, 1090.
NEGELEIN, E. 1925. Biochem. Zeitschr., clxv, 203.
NENCKI, M. 1900. Arch. Exp. Pathol., xxiv, 442.
OPPENHEIMER, C. 1909. Handbuch der Biochemie, i, 709.

PHILLIPI, E. 1919. Zeitschr. Physiol. Chem., civ, 88.
PREGL, F. 1905. Zeitschr. Physiol. Chem., xliv, 173.
ROBINSON, M. E. 1924. Biochem. Journ., xviii, 255.
ROCHE, J. 1929. Arch. d. Physique Biol., vii, 165.
SCHULZ, F. N. 1898. Zeitschr. Physiol. Chem., xxiv, 449.
SCHUMM, O. 1925. Zeitschr. Physiol. Chem., cl, 276.
1926a. Zeitschr. Physiol. Chem., clii. 55.
1926b. Zeitschr. Physiol. Chem., clii, 147.
1926c. Zeitschr. Physiol. Chem., cliv, 171.
SORBY, H. 1876. Quart. Journ. Micr. Sci., xvi, 76.
STENGER, F. 1888. Wiedem. Ann., xxxiii, 577.
STOKES, G. 1863–4. Proc. Roy. Soc., xiii, 355.
SVEDBERG, T. 1909. Koll. Zeitschr., v, 318.
TREIBS, A. Z. 1927. Zeitschr. Physiol. Chem., clxviii, 68.
WARBURG, O. 1919. Biochem. Zeitschr., c, 268.
1926. Biochem. Zeitschr., clxxvii, 471.
1927. Biochem Zeitschr., clxxxix, 354.
1928. Ueber die Katalytischen Wirkungen der Lebendigen Substanz., Berlin.
1929a. Biochem Zeitschr., ccxiv, 1.
1929b. Biochem. Zeitschr., ccxiv, 4.
1929c. Zeitschr. f. Elektrochem., xxxv, 549.
WARBURG, O., UND F. KUBOWITZ. 1928. Biochem. Zeitschr., cciii, 95.
1929a. Biochem. Zeitschr., ccxiv, 5.
1929b. Biochem. Zeitschr., ccxiv, 19.
1929c. Biochem. Zeitschr., ccxiv, 24.
WARBURG, O., UND E. NEGELEIN. 1928a. Biochem. Zeitschr., cxciii, 334.
1928b. Biochem. Zeitschr., cxciii, 339.
1928c. Biochem. Zeitschr., cc, 414.
1928d. Biochem. Zeitschr., ccii, 202.
1929a. Biochem. Zeitschr., cciv, 495.
1929b. Biochem. Zeitschr., ccxiv, 64.
1929c. Biochem. Zeitschr., ccxiv, 101.
1929d. Zeitschr. Elektrochem., xxxv, 928.
WARBURG, O., E. NEGELEIN UND W. CHRISTIAN. 1929. Biochem. Zeitschr., ccxiv, 26.
WILLSTÄTTER, R. UND M. FISCHER. 1912. Zeitschr. Physiol. Chem., lxxxvii, 423.
WILLSTÄTTER, R. UND A. STOLL. 1913. Untersuchungen über Chlorophyll. Berlin.
WEHMER, C. 1925. Ber. Deutsch. Botanges., xliii, 184.
WU, H. 1926. Colloid chemistry. Ed. by J. Alexander, New York, 1, p. 380.
YAOI, H. AND TAMIYA. 1928. Proc. Imp. Akad (Tokyo) iv, 436.
AND W. NAKAHARA. 1928. Proc. Imp. Akad. (Tokyo), iv, 443.
v. ZEYNEK, R. 1898. Zeitschr. Physiol. Chem., xxv, 492.
1910. Zeitschr. Physiol. Chem., lxx, 224.